A CUTANEOUS REACTION IN SYPHILIS.*

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PLATES 50–52.

Active or passive immunity, the state of allergy, and numerous other phenomena, which have been so commonly observed and profitably studied in certain infectious diseases since the successful pure cultivation of their respective causative agents, are rather imperfectly understood in syphilis. Soon after obtaining pure cultures of *Treponema pallidum,* I commenced to study these questions experimentally. While the results of my experiments on the effects of repeated injections of living and killed pallida on experimental syphilis in animals will be presented later, I wish to make a brief report here on one of the phases of study that seems to me to be of interest not only to those who are engaged in experimental work but also to clinicians at large; namely, on a cutaneous reaction in experimental and human syphilis, to the possible existence of which my attention was directed by Professor Welch.

Stimulated by von Pirquet's discovery of a specific cutaneous reaction for tuberculosis, several investigators (Meirowsky, Wolff-Eisner, Tedeschi, Nobl, Ciuffo, Nicolas, Favre and Gauthier, Neisser and Bruck, and Jadassohn) attempted to obtain a specific reaction for syphilis by applying extracts of syphilitic tissues, prepared from syphilitic fetal liver or chancre, to the skin of syphilitic patients. Their results were, on the whole and in spite of some encouraging effects, contradictory. Neisser and Bruck, however, found that a reaction similar to that produced with syphilitic extract can be obtained also with a concentrated extract of normal liver. This peculiarity of the skin of syphilitics is ascribed by Neisser to what he calls the state of "Umstimmung" in the later stages of

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syphilis. Neisser, nevertheless, expresses the hope that the reaction may possibly be improved by employing an extract of the pallidum free from tissue constituents. This had not been accomplished before. Pure cultures of *Treponema pallidum* obviously offer many advantages, since in them we possess not only pallida of different ages, but also their metabolic products, which doubtless are important factors in establishing an allergic state in syphilitic subjects.

THE PREPARATION OF CULTURES FOR THE CUTANEOUS REACTION.

Two strains of the pallidum were used for the present series of experiments. Pure cultures were allowed to grow for periods of six, twelve, twenty-four, and fifty days at 37° C. under anaerobic conditions. One was cultivated in ascitic fluid containing a piece of sterile placenta, and the other in ascitic fluid agar also containing placenta. The lower portion of each solid culture in which a dense growth had occurred was cut out and the tissue removed. The agar columns which contained innumerable spirochætæ were then carefully ground in a sterile mortar. The resulting thick paste was gradually diluted by adding, little by little, the fluid culture which also contained an enormous mass of the pure organisms. The dilution was continued until the emulsion became perfectly liquid. The preparation was next heated to 60° C. for sixty minutes in a water bath, and then 0.5 per cent. carbolic acid was added. When examined under the dark-field microscope, 40 to 100 dead pallida per field could be seen. Cultures made from this suspension remained sterile, and with them no infection could be produced in the testicles of rabbits. The suspension was kept in a refrigerator when not in use. I propose to speak of this preparation under the name of *luetin*.

In order to ascertain whether the reaction produced with this suspension might not be due to the introduction of the carbolized culture medium alone, it was necessary to prepare a similar emulsion with uninoculated media to be used for control purposes.

METHOD OF APPLICATION.

For rabbits, the hair on the lower side of the back is shaved off and one or two injections of the luetin and one injection of the

control suspension are made in different spots. The injections are made intradermically with a very fine needle, the amount injected being in each case 0.05 of a cubic centimeter. For human subjects, the skin of the upper arm was selected as the site for the intradermic injection of the luetin and the control suspension. In the earlier experiments, different spots on the same upper arm were used for both, but later, one arm was used for luetin and the other for the control. The amount injected in each instance was 0.05 of a cubic centimeter, and the skin was always sterilized with alcoholic sublimate solution before the injection.

THE CUTANEOUS REACTION IN RABBITS.

Before the skin test was applied to human subjects, several series of experiments were performed on rabbits. The tests may be divided into five groups according to the treatment previous to the cutaneous tests. The first group comprised four rabbits which received within five months twelve intravenous injections of emulsions prepared from syphilitic orchitis or rabbits rich in pallida, the last injection being given two months before the date of application of the skin reaction. Ten different strains of pallidum were represented in this series. The second group was composed of four rabbits which had been injected with similar emulsions, the difference being that the pallidum had been killed by heating the emulsions to 60° C. for thirty minutes before injection. The third group included twelve rabbits which were showing, four to six weeks after inoculation, syphilitic orchitis experimentally produced with several different pallidum strains. The fourth group consisted of four rabbits in which experimental syphilitic orchitis had been cured about four months previously by the intravenous administration of salvarsan. The fifth group consisted of eight normal male rabbits. The intradermic injections of the luetin and control emulsions were made on the same day to all the rabbits.

RESULTS OF THE TESTS.

In the control group, a very slight erythematous condition appeared at the injection sites twenty-four hours after inoculation, which disappeared within forty-eight hours, the skin resuming its

normal appearance. The first and second groups of treated rabbits gave different reactions. The sites of injection of the control emulsion became normal within forty-eight hours, while the sites of injection of the luetin became distinctly red and indurated after forty-eight hours. The point of redness gradually spread and became raised to the size of a pea. This condition persisted for four to six days, when the reaction commenced to recede. The redness and induration disappeared in most animals within ten days. In one instance, a round sterile pustule developed on the fifth day on the site of each of the two inoculations of the luetin (figure 1). These pustules resembled variolous pustules in general appearance and healed with crust formation. The third group gave only slight reactions, which possibly were somewhat greater than in the control rabbits. But, as a whole, they were not, except in a few instances, distinct enough to be considered positive. The fourth group behaved like the control. No constitutional effects were observed even among the animals with positive cutaneous reactions.

THE CUTANEOUS REACTION IN MAN.

Through the courtesy and collaboration of Dr. Martin Cohen (Harlem Hospital, Randall's Island Asylum, New York Ophthalmic and Aural Institute), Dr. Henderson (State Hospital, Ward's Island, New York), Dr. Lapowski (Good Samaritan Dispensary), Dr. McDonald (King's County Hospital), Dr. Orleman-Robinson (North-Western Clinic, New York Polyclinic), Dr. Pollitzer (German Hospital), Dr. Rosanoff (King's Park State Hospital), Dr. Satenstein (City Hospital, Blackwell's Island, New York), Dr. Schmitter (Captain United States Army, at Fort Slocum), Dr. Schradieck (King's County Hospital), Dr. Charles Schwartz (California), Dr. Smith (Long Island State Hospital), Dr. Strong (Manhattan Eye, Ear, and Throat Hospital), Dr. Swinburn (Good Samaritan Dispensary), Dr. Windfield (King's County Hospital), Dr. Wiseman (King's Park State Hospital), and the Hospital of the Rockefeller Institute for Medical Research, I was enabled to apply the skin reaction to a number of human cases.

The total number of cases studied was 400; of these 177 were of syphilitic nature, 77 of parasyphilitic nature, while 146 represented various controls.

The emulsions were always injected intradermically, that is, in the skin as superficially as possible. When the injection is successfully carried out, the epidermic layer is raised up sharply from the cutis, the pale swelling thus produced going down in from ten to fifteen minutes.

DESCRIPTION OF THE REACTIONS.

Normal or Negative Reactions.—After applying the emulsions, both luetin and control, to about fifty normal individuals, I was able to determine the variations and limitations of the reactions that follow intradermic administration in the normal skin of man. In the majority of normal persons, there appears, after twenty-four hours, a very small erythematous area at and around the point of injection. No pain or itching sensation is experienced. This reaction gradually recedes within forty-eight hours and leaves no induration. In certain individuals, the reaction may reach a stage of small papule formation after twenty-four to forty-eight hours, after which and within seventy-two hours it commences to subside. No induration is left behind, although occasionally slight yellowish pigmentation may result from mild ecchymosis.

Positive Reactions.—According to the manner and intensity with which the skin of syphilitics responds to the introduction of the luetin, one may distinguish the following varieties of effects:

(*A*) *Papular Form.*—A large, raised, reddish, indurated papule, usually five to ten millimeters in diameter, makes its appearance in twenty-four to forty-eight hours. The papule may be surrounded by a diffuse zone of redness and show marked telangiectasis. The dimensions and the degree of induration slowly increase during the following three or four days, after which the inflammatory processes begin to recede. The color of the papule gradually becomes dark bluish red. The induration disappears within one week, except in certain instances in which a trace of the reaction may persist for a longer period. This latter effect is usually met with among cases of secondary syphilis under regular mercurial treatment in which there are no manifest lesions at the time of making the skin test. Cases of congenital syphilis also show this reaction.

(*B*) *Pustular Form.*—The beginning and course of this reaction resemble the papular form until about the fourth or fifth day, when

the inflammatory processes commence to progress. The surface of the indurated, round papule becomes mildly edematous, and multiple miliary vesicles occasionally form (figure 2). At the same time, a beginning central softening of the papule can be seen. Within the next twenty-four hours, the papule changes into a vesicle filled at first with a semi-opaque serum that later becomes definitely purulent (figures 3 and 4). Soon after this, the pustule ruptures spontaneously or after slight friction or pressure. The margin of the broken pustule remains indurated, while the defect caused by the escape of the pustular content becomes quickly covered by a crust that falls off within a few days. About this time the induration usually disappears, leaving almost no scar after healing. There is a wide range of variation in the degree of intensity of the reaction described in different cases, as some show rather small pustules, while in others the pustule is much larger. This reaction was found almost constantly in cases of tertiary syphilis, as well as in cases of secondary or hereditary syphilis which had been treated with salvarsan.

(*C*) *Torpid Form.*—In rare instances, the injection sites fade away to almost invisible points within three or four days, so that they may be passed over as negative reactions. But sometimes these spots suddenly light up again after ten days or even longer and progress to small pustular formation. The course of this pustule is similar to that described for the preceding form.

This form of reaction has been observed in a case of primary syphilis, in one of hereditary syphilis, and in two cases of secondary syphilis, all being under mercurial treatment.

Neither in syphilitics nor in parasyphilitics did a marked constitutional effect follow the intradermic inoculation of the luetin. In most positive cases, a slight rise in temperature took place lasting for one day. In three tertiary cases and in one hereditary case, however, general malaise, loss of appetite, and diarrhea were noted.

THE PECULIARITY OF THE SKIN OF SYPHILITICS.

The skin of certain individuals suffering from tertiary syphilis is noted for its susceptibility to traumatic irritations. This tendency has been much discussed by dermatologists. To account for this

phenomenon, Finger advanced the theory of superinfection, which presupposes that a trauma creates a spot of weakened resistance in the skin and that the syphilitic virus wanders thither from a hidden focus in the interior to set up the lesion. On the other hand, Neisser maintains that it is not the spirochæta that produces a syphilitic lesion after a slight trauma, because he failed to prove the presence of any spirochæta or infectious agent in the lesions; but he believes that it is due to a pathological condition of the skin itself, which he calls "Umstimmung." Among 177 cases of syphilis, I observed nine cases (one secondary, six tertiary, and two hereditary) in which the sites of the control emulsion reacted quite intensely. But these reactions were always less intense than those produced by the leutin in the same persons. Furthermore, in these cases the induration remained much longer at the sites of luetin injection than at the control sites. In view of these results and of the additional fact that the majority of cases giving the positive skin reactions did not react to the control emulsion, I am of the belief that the reactions set up by the intradermic inoculation of luetin are due not to Neisser's so-called state of "Umstimmung," but to a purely allergic condition. It is, of course, possible that both conditions may be coëxistent in the same patient, although the occurrence of "Umstimmung" appears, from my experience, to be far less frequent than that of allergy. In carrying out the cutaneous reaction for syphilis (as for any other infectious disease), it is highly important to provide adequate control observations to determine whether the state of "Umstimmung" exists only in syphilis, or whether it may not also occur in other diseases.

THE RESULTS OF THE CUTANEOUS REACTION IN MAN.

In all, 400 cases, comprising 177 syphilitics, 77 parasyphilitics, and 146 controls, have been studied. The results obtained in syphilitics are summarized in table I. The table shows that in cases of primary and secondary syphilis, which have had either insufficient treatment or none at all, no skin reaction occurred, except in a few instances. The reaction in the positive cases was always of the indurated papular variety. In one primary case, in which the skin test was applied before regular mercurial treatment was begun, a

torpid reaction followed the administration of mercury. On the other hand, most of the secondary cases which had been mercurialized before the administration of salvarsan, and which remained

TABLE I.

	Primary syphilis.		Secondary syphilis.				Tertiary syphilis.				Cerebro-spinal syphilis.		Hereditary syphilis.		Latent syphilis.		Controls.			
	Symptoms present.		Symptoms present.		Symptoms absent.		Symptoms present.		Symptoms absent.		Symptoms present.		Symptoms present.				Normal individuals.		Non-luetic cases.	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
No antisyphilitic treatment		2													24	6		46		100
Slight mercurial treatment	1	2	2	10			12													
Regular antisyphilitic treatment.....					4	3	15		19	1	5	5	19	1						
Salvarsan treatment.....			1		22	8			11	1			3							
	1	4	3	10	26	11	27	0	30	2	5	5	22	1	24	6	0	46	0	100
	5		50				59				10		23		30		146			
	177																			

\+ indicates a positive, and — a negative cutaneous reaction.

without symptoms for some months after the salvarsan injections, gave striking and unmistakable reactions.

Two distinct groups that give a negative or doubtful reaction occur among the salvarsan cases. One consists of cases showing a reappearance of the Wassermann reaction, but no other symptoms, and usually gives doubtful cutaneous reactions. The other, which, however, comprised only two cases treated with salvarsan that remained without any clinical symptoms or Wassermann reaction for many months, gave no cutaneous reaction.

To this group also belong two interesting cases that have been under my observation since September, 1910. One was treated for gumma of the liver by an intramuscular injection of salvarsan by Dr. Kakels at the Lebanon Hospital. The large gumma promptly disappeared, and the patient has remained without symptoms up to the present. The Wassermann reaction in this patient disappeared slowly but completely in about ten weeks. The

blood, tested every two months, has remained negative. The skin test with luetin, made in August, 1911, was negative. The other case is one of malignant syphilis, refractory to mercurial treatment. Three intramuscular injections of salvarsan were given in September and November, 1910, and in January, 1911. The Wassermann reaction disappeared soon after the healing of the extensive and destructive lesions, and still remains negative. A luetin test made in August, 1911, was negative. Both patients are now in excellent health. I have introduced these cases to illustrate the point that the allergic state of the skin in syphilis seems not to persist beyond a certain time after the probable complete eradication of the pallidum from the body. This point has been confirmed experimentally by rabbits treated with salvarsan.

The results in tertiary and hereditary syphilis present a striking contrast to those observed in the earlier stages of the disease. The skin reacts intensely to luetin in these cases. The intensity of the reaction in the tertiary cases is probably increased by the state of "Umstimmung," but the constancy with which it appears suffices to render it a valuable clinical diagnostic aid. It is in this stage that syphilis manifests itself in its most diverse and often most obscure forms. A gumma or other tertiary lesion on the visible parts of the body offers but little diagnostic difficulty; but it is not always easy to ascertain the luetic nature of lesions of the internal organs. In tertiary cases, moreover, the Wassermann reaction is frequently absent, especially after specific treatment. Thus it is important to find that the skin reaction is more constant than the Wassermann reaction in tertiary syphilis. Two causes for a negative Wassermann reaction in the tertiary stage may be distinguished. The first, which may apply also to latent syphilis, consists of the restraining power of the body upon the propagation of the pallidum together with the neutralization of its injurious products through the formation of antibodies. The second cause, which also operates in some primary and secondary cases, arises from the inability of the body to respond to the pallidum stimulus by the formation of the substances on which the Wassermann reaction depends. The results of the luetin reaction in syphilis of the central nervous system are even less constant than is the Wassermann reaction.

Whether a better result can be obtained by improving the luetin can be determined only by future work. The cases of latent syphilis reported here consist chiefly of parents of the hereditary syphilitic cases reported in table I. Most of them became infected many years before and have remained without symptoms. Many of the mothers have, however, suffered miscarriages previous to or after the birth of their syphilitic children.

The controls include 46 normal individuals, chiefly children between the ages of two and eighteen years, and 100 individuals suffering from various diseases of non-syphilitic nature. These include tuberculosis, leprosy, pneumonia, typhoid fever, psoriasis, malaria, alcoholic psychosis, dementia præcox, gonorrhea, chancroid, brain tumor, eczema, epithelioma, carcinoma, etc. In none was a positive luetin reaction obtained.

The results thus far obtained in parasyphilitic cases are unsatisfactory and call for further study with more active preparations of the luetin. Seventy-two cases of general paralysis and five of tabes were studied. Of the seventy-two cases of general paralysis, forty-five reacted positively. In a few instances pustules were formed, but twenty-seven gave no reaction. Of five cases of tabes, three reacted positively. Among thirty-five controls of this series, consisting of fifteen cases of dementia præcox, six of alcoholic psychosis, and fourteen of other non-syphilitic psychoses, four reacted positively, and these were cases of dementia præcox. These patients were adults, and two of them gave positive Wassermann reactions. I incline, therefore, to the opinion that they had suffered either from congenital or acquired syphilis.

CONCLUSIONS.

1. I have proposed the name *luetin* for an emulsion or extract of pure cultures of *Treponema pallidum* which is designed to be employed for obtaining, in suitable cases, a specific cutaneous reaction that may become a valuable diagnostic sign in certain stages or forms of syphilitic infection.

2. The repeated inoculation of either living or killed *pallida* into the testicles of rabbits leads to a condition in which an intradermic injection of luetin is followed by a well marked inflammatory reac-

tion. A corresponding reaction has been obtained neither in rabbits suffering from active syphilitic orchitis, nor in those in which the condition had been cured by the administration of salvarsan four months previously. Normal rabbits, likewise, do not react to the luetin.

3. The luetin produces a similar cutaneous reaction in syphilitic and parasyphilitic patients that is most constant and severe in the tertiary and hereditary affections. In my series of cases, it was present constantly (100 per cent.) in the manifest tertiary affection, in 94 per cent. of latent tertiary affection, and in 96 per cent. of the hereditary affection.

4. During the primary and secondary stages, the reaction is infrequent, and when present it is of mild degree. An exception has been found in cases in which energetic treatment has been or is being carried out and in which clinical signs of syphilis are absent. Such cases may show a severe reaction. Apparently this is true especially of the cases treated with salvarsan.

5. In certain cases of old infection in which no treatment has been taken and in which no symptoms have appeared for many years, and in the course of which miscarriages have not occurred, the cutaneous reaction has failed to appear. But, despite the absence of symptoms, mothers who have young syphilitic children have usually given the reaction.

6. It remains to be determined in how far the cutaneous reaction with luetin can be used to supplement the Wassermann reaction in determining the complete and permanent suppression of a syphilitic affection.

7. It appears probable that the Wassermann reaction is more constant in the primary and secondary, and the cutaneous reaction in the tertiary and latent forms of syphilis. Moreover, it appears that the Wassermann reaction is more directly and immediately affected by antisyphilitic treatment than is the cutaneous reaction.

8. A more active preparation of luetin can certainly be produced by improved methods. This phase of the subject is being considered at the present time and will be reported upon in a later paper.

EXPLANATION OF PLATES.

PLATE 50.

FIG. 1. An actively immunized rabbit (No. 4), inoculated on June 14th, 1911, and painted on the sixth day. Both pustules are produced by the luetin. The control site disappeared.

FIG. 2. Female, aged 16 years. Hereditary syphilis, and interstitial keratitis of six months' standing. Salvarsan injected five months ago without good results. Wassermann reaction positive. Inoculated on June 17th, 1911, and painted on the fifth day. Both sites of the luetin injection show a tendency to pustulation. Marked telangiectasis.

PLATE 51.

FIG. 3. Male, aged 29 years. Chancre seven months ago, followed by secondaries. Six intramuscular injections of salvarsan, the last about three months ago. Developed exudative choroiditis six weeks ago. The right eye shows typical exudative choroiditis; the left eye is free from it. Wassermann reaction negative. Inoculated on June 23, 1911, and painted on the sixth day. Between the two large luetin pustules there is a faint trace of the injection of the control material.

PLATE 52.

FIG. 4. Female, aged 51 years. Became infected twenty years ago, and was treated moderately by Lassar in Berlin. Present symptoms: exostosis and stenosis of lacrimal canal. Wassermann reaction weakly positive. Luetin test on July 11th. The left arm shows two well marked pustules at the sites of the luetin injection, while the right arm shows a faintly visible trace on the control site. Photographed on the sixth day. (Dr. S. Pollitzer kindly took this photograph for me.)

FIG. 2.

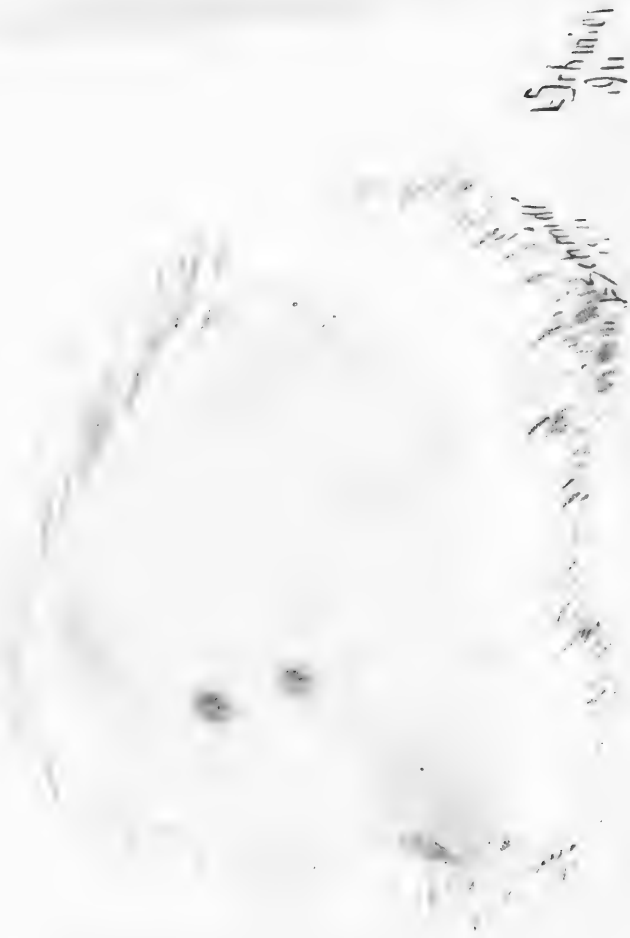

FIG. 1.

FIG. 3.

LEFT. RIGHT.

FIG. 4.

THE STATUS OF RESPIRATION IN THE METHODS OF DIFFERENTIAL PRESSURE COMPARED WITH THAT UNDER THE METHOD OF INTRA-TRACHEAL INSUFFLATION.*

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INTRODUCTION.

The methods of so-called differential pressure which were introduced several years ago into experimental and practical surgery of the thoracic cavity, contain an obscure point which is in need of elucidation. All the present writers on the subject of differential pressure seem to assume as granted that the danger of the open thorax is brought about by the collapse of the lungs, and, since under-pressure as well as over-pressure keeps the lungs inflated, nobody seems to find it surprising that both these methods are capable of obviating this danger. This assumption, however, is based upon a confusion of two independent sequences to one and the same cause, and is entirely erroneous. It is true that the lungs in the normal thorax fill out the entire cavity, and it is also true that the opening of the cavity leads (1) to a collapse of the lungs and (2) to dyspnea and death. But it is a mere confusion, very widely spread, of two facts to assume that the dyspnea and death are due to the collapse of the lungs. Dyspnea and death are caused by the circumstance that the respiratory changes in the volume of the thoracic cavity are now compensated through the artificial openings in the thorax and not by the way of the normal respiratory paths. Simple collapse of the lungs need not lead to death. A simple experiment will demonstrate this. Take for instance the dog, an animal that usually succumbs to a one-sided pneumothorax. If the pleura is opened widely on one side, the

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corresponding lung collapses to a considerable degree, and frequently the respiration soon becomes very labored and dyspneic; the life of the animal is then in immediate danger. But now close the opening in the thorax tightly by the skin flap, or by some other efficient method, and the animal quiets down immediately and normal respiration prevails in a minute or two. The air is not aspirated from the pleural cavity, nor could it be absorbed in this short time; it is still there and keeps the lung collapsed; nevertheless, the animal is restored to rest and tranquil breathing. It is evident that it was not the presence of air in the thoracic cavity and the consequent collapse of the lung which caused the stormy, dangerous manifestations. It was the wide opening in the thorax that caused the trouble, the inspiratory distension of the thoracic cavity aspired air into the pleural cavity through the opening in the thoracic wall instead of through the trachea into the lungs, and the expiratory compression of the chest drove out air from the pleural cavity instead of the vitiated air from the lungs. Only an open pneumothorax causes dyspnea; when the cavity is closed, even though the lungs have been recently collapsed, an inspiration will still cause some distension of the lungs and an aspiration of air into them, and an expiration will cause a compression of the lungs and a removal of the carbon dioxid-laden air. In experiments on rabbits, Hellin[1] noted that some of the animals survived even after the collapse of both lungs (double pneumothorax), when the opening of the second pleural cavity was made after the opening into the first was closed. The same author collected, a few years ago,[2] from the surgical literature a large number of clinical cases of patients who survived a double pneumothorax. We may recall here the fact that in recent years the attempt has variously been made to institute a one-sided pneumothorax as a therapeutic means in certain conditions of pulmonary tuberculosis. Murphy,[3] who has been among the first to recommend this method of treatment, insisted that "the pneumothorax, *per se,* does absolutely no harm," and that "it is the absence of respiratory change

[1] Hellin, *Berl. klin. Wchnschr.,* 1901, xxxviii, 1020.
[2] Hellin, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.,* 1907, xvii, 414.
[3] Murphy, *Jour. Am. Med. Assn.,* 1908, li, 814.

that makes the condition a dangerous one." Sauerbruch,[4] in his extensive studies on the pathology of the pneumothorax which led up to the development of his under-pressure method, seemed to have come to the conclusion that, indeed, the collapse of the lung is the cause of the dyspnea of the one-sided pneumothorax; but, in his opinion, the dyspnea is caused not by the elimination of the respiratory capacity of the collapsed lung, but by the fact that a good deal more blood flows through the vessels of a collapsed lung than through a normally distended one and that in consequence in a one-sided pneumothorax an insufficient volume of blood flows through the lung in the closed pleural cavity. That this view is incorrect is shown by Seidel,[5] who clamped the artery of the collapsed lung without bringing out any change in the condition of the dyspnea. Sauerbruch attaches much importance also to the elimination of the vagus reflex in the collapsed lung which normally controls the respiratory tonus. We may remind the reader here of the fact that section of the vagus on one side exerts practically no effect upon the respiration. It is noteworthy that Sauerbruch apparently has not availed himself of the above mentioned simple experiment of closing the opening; it would have demonstrated to him that the dyspnea is due to the thorax being open and not to the collapsed condition of the lung.

We may say, however, that it was fortunate for us that Sauerbruch entertained the belief in the danger of the collapse of the lungs. Otherwise he would not have conceived the idea, and consequently not have discovered the fact, that a stationary distension of the lungs is capable of keeping the animal alive and free from dyspnea, even when both pleural cavities remain wide open. And now we come to the obscure point mentioned at the beginning of this discussion. For clearness' sake, let us recapitulate briefly our foregoing analysis. The opening of the thorax causes two conditions—the collapse of the lungs and the establishment of an exchange of air through the wide opening in the thoracic wall instead of through the lungs. It is evident, on the one hand, that the dyspnea and the danger which opening of the thorax brings

[4] Sauerbruch, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1904, xiii, 399.
[5] Seidel, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1907, xvii, 668.

about come essentially, if not exclusively, from the latter condition. On the other hand, we are confronted with the undeniable fact that a simple redistension of the lungs, be it by under-pressure or over-pressure, abolishes the dyspnea and sustains the life of the operated animal or man, even if both pleural cavities remain widely open. The point which now demands elucidation is the question, How can the mending of the collapse of the lungs be of any assistance to the difficulties of the respiration arising from the free access of air to the pleural cavities? Even with widely distended lungs, with each inspiratory distension of the chest cavity, air will rush with incomparably greater facility through the wide opening on the chest than into the lungs. The same is true for expiration; the air will rush out more readily from the wide artificial opening of the pleural cavities than from the lungs. How, then, is the proper exchange of gases within the lungs accomplished by the aid of the methods which seem to accomplish nothing but a certain degree of constant distension of the lungs? With the exception of Sauerbruch, none of the numerous writers on the problems of differential pressure, has, to our knowledge, even as much as touched upon this problem. Sauerbruch, however, was so profoundly impressed with the difficulty that he was at first inclined to accept the notion that the lungs are capable of distending and collapsing by a neuro-muscular mechanism of their own, and that the respiratory mechanism of the thorax was merely a non-essential accessory. This idea Sauerbruch discarded, however, after finding that the respiratory movements of the lungs ceased completely after removal of the entire thorax.

Sauerbruch's final explanation of the puzzling problem we shall mention later, after reporting the results of our own experiments which have some bearing upon this question.

EXPERIMENTAL OBSERVATIONS.

From the surgical literature, we gather the impression that intrathoracic operations under differential pressure, those performed upon the inferior lobes of the lungs or upon the lower part of the esophagus, seem to have had a decidedly greater mortality than operations performed upon parts lying in the middle or upper part

of the thorax. On the basis of the hypothesis that the normal position of the inferior lobes of the lungs is an essential requirement for the success of differential pressure, we carried out a number of experiments in which the relations of the lower lobes were interfered with. The effect of the procedure was tested, in parallel experiments, under the Brauer method of over-pressure and under our method of intratracheal insufflation.

Methods.—The observations were made on dogs which were under the influence of ether anesthesia throughout the entire experiment. The over-pressure procedure was the same as that employed on animals by Brauer.[6] One branch of a T tube was introduced into the trachea of the animal and the other was connected with the tank of an air pump, which thus sent a stream of air into the trachea. The third branch of the T tube was connected with a piece of rubber tubing, the free end of which was submerged under about ten to fifteen centimeters of water. The air stream was in most cases continuous—as is the rule in Brauer's method. In a few instances, the stream was interrupted about twenty times per minute.

Regarding the details of the insufflation method, we shall refer to a previous paper on the subject.[7] We shall expressly mention that the air stream was interrupted about ten times per minute, the interruptions being of short duration and permitting only a partial collapse of the lungs.

The incisions were made in the fifth, sixth, or seventh intercostal spaces. In no case was the sternum transected or the ribs resected; but the entrances into the pleural cavities were made fairly wide.

Results.—Of the nineteen dogs which were subjected to a double pneumothorax under the Brauer method, eighteen continued to live in a normal state of respiration until they were otherwise experimentally interfered with. One dog died under symptoms of severe dyspnea sixteen minutes after the left pleural cavity alone was opened. The Brauer method proved in our experiments practically to be as efficient as is generally claimed for it. The chief result obtained in our experiments consists in the practically and theoretic-

[6] Brauer, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1904, xiii, 483.
[7] Meltzer, *Med. Rec.*, 1910, lxxvii, 477.

ally important fact that a complete dislocation of the lower lobes of the lungs leads invariably to a fatal termination under the Brauer method, while it has no detrimental effect under our method of insufflation. This fact as well as a few other results of varying importance are well illustrated by some abbreviated protocols of experiments which we shall reproduce here.

Experiment 1.—Dog, weight 14 kilos. Brauer method; constant air stream under pressure of 15 mm. of mercury; lateral tube under 15 cm. of water.

12:10. Both vagi exposed in the neck; intact.

12:15. Respiration 72, pulse 144. Stimulated left vagus with induction current (1 Daniell, Petzold apparatus). At 40 mm. coil distance, slowing of heart; at 30 mm., at first complete inhibition, later escape and slowing.

12:24. Left chest, and 12:29, right chest widely opened in fifth intercostal space.

12:30. Respiration 44, pulse 140 per minute; regular.

12:42. Stimulated left vagus, intact; 80 mm. coil distance, no effect; 60 mm., strong stoppage of heart with escape.

12:44. Both vagi cut.

12:49. Respiration 21, heart 220 per minute.

12:53. Stimulated peripheral end of left vagus; 80 mm. coil distance, strong stoppage, followed by marked slowing; 100 mm. coil distance, no effect.

12:54. Hand introduced into chest, posterior mediastinum torn, both lower lobes of lungs freed and delivered outside of the chest through the corresponding openings.

12:57. Respiration stopped, heart feeble.

12:59. Heart stopped.

In this experiment, an animal with an open double pneumothorax under an over-pressure arrangement continued to be apparently in a normal state for twenty-five minutes. However, after freeing both lower lobes and pulling them into the openings of the thorax, the respiration stopped after three minutes, and the heart stopped two minutes later, although on account of the presence of the lungs in the openings the exchange of air through these operations must have been somewhat restricted. Death could not have been due to reflex action on either heart or respiration, since both vagi were cut before the lower lobes were freed. It should be noticed that the *respiration stopped before the complete failure of the heart.* Another point worth noticing in this experiment is the fact that very effective stimulations of the intact vagi had no detrimental after-effect upon the animal as long as the lower lobes were in their normal position.

In this experiment, the lower lobes were pulled out soon after the vagi were cut. It could be claimed that the immediately preceding cutting of the vagi may have contributed to the fatal issue. In the following experiment, the dislocation of the lungs took place in an animal with intact vagi.

Experiment 2.—Male dog, weight 12,000 grams. Brauer method; continuous air stream under pressure of 10 to 15 mm. of mercury and 12 cm. pressure of the water valve; vagi intact.

10:44. Left chest, and 10:55, right chest opened widely in sixth intercostal space.

11:08. Heart beats 120, respiration 45 per minute.

11:14. Air stream interrupted for 30 seconds, lungs collapsed: *heart slowed swiftly.* Air stream resumed; *no respiration for a number of seconds, then slow and labored.*

11:16. Heart 83, respiration 22 per minute.

11:22. Heart 105, respiration 39 per minute.

11:47. Heart 114, respiration 45 per minute.

11:49. Interrupted air stream for 30 seconds: *powerful slowing of heart at once.*

11:52. Heart 93, respiration 33.

11:55. Lower lobes of lungs taken outside of chest and held there with clamps at border of lobes. Respiration became labored at once.

11:57. Heart 42, respiration 6 per minute, very labored.

12:00. Heart 27, respiration 4 per minute.

12:08. Heart 69 per minute, feeble, irregular. No respiration.

12:12. No heart beats.

In this experiment, the lower lobes of the lungs were pulled out of the chest one hour after the establishment of the double pneumothorax. Heart and respiration continued to all appearances to be normal. As soon as the lower lobes were held in the openings of the thorax, the respiration became labored and slow; it stopped completely after thirteen minutes, and the heart stopped four minutes later. We call attention to the fact that in this experiment an interruption for only thirty seconds had a considerable effect; it slowed the heart powerfully, practically at once, and both heart and respiration did not return to normal for many minutes.

We shall say that in this experiment as well as in the others the dislodged lungs remained distended and that only a small part of them was outside of the thoracic cavity; it was often necessary to apply clamps to these lobes to prevent them from slipping back to their natural moorings.

Experiment 3.—Dog, weight 20 kilos. Brauer method; constant air stream under pressure of 18 mm. of mercury and 12 cm. water pressure.

10:43. Left side of thorax, and 10:49, right side, widely opened.

11:57. Heart beats 130, respiration 63 per minute.

11:59. Both lower lobes delivered outside of chest wall.

12:06. Heart 116, respiration labored, 76 per minute. Lower lobes frequently fall back into position and have to be pulled out again.

12:15. Respiration stopped; heart weak, 9 beats per minute.

12:17. Again a few convulsive respirations.

12:18. Dead.

In this experiment, the animal survived the first pulling out of the lower lobes by nineteen minutes, but the lobes frequently slipped back into their position and had to be pulled out again. This maneuver acted apparently as a sort of artificial respiration and assisted somewhat in the prolongation of life. At first the procedure of delivery seemed even to accelerate temporarily the respiration. In this experiment, the dislocation of the lung was instituted fairly soon after the opening of the thorax.

Experiment 4.—Dog, weight 8 kilos. Brauer method; constant air stream under pressure of 15 mm. of mercury and 15 cm. for the water valve.

2:16. Both pleural cavities opened widely.

2:18. Heart 90, respiration 36 per minute.

2:22. Both vagi cut.

2:41. Heart 144, respiration 10 per minute.

2:42. Both lower lobes pulled outside of chest.

2:48. No respiration, heart 120, getting feeble.

2:49. No femoral pulse, no respiration.

2:50. No pulse.

The cutting of the vagi, which in this experiment, as in any of the other experiments, slowed the respiration, had for twenty minutes apparently no deleterious effect upon the animal. The pulling out of the lungs, however, stopped the respiration completely in six minutes and the heart in eight minutes.

Experiment 5.—Male dog, weight 2,650 grams. Brauer method; constant air pressure of 20 mm. of mercury, and 15 cm. pressure of water valve.

10:30. Both pleural cavities opened. Vagi intact.

10:35. Heart 140, respiration 104 per minute.

10:45. Stimulated right vagus (intact) with 80 mm. coil distance; strong slowing of heart beats; respiration stopped during stimulation.

10:46. Rubbing pleura on both sides with finger: no effect upon heart.

11:30. Heart 148, regular; respiration 76 per minute.

11:35. Stimulated left (intact) vagus with 40 mm.; heart and respiration stopped.

11:40. Stimulated various parts of pleura with induced current at 40 mm. coil distance; no effect upon heart, practically no effect upon respiration.

11:41. Both vagi cut.

12:00. Respiration 20, heart 132, regular, good. Anterior mediastinum torn and both lower lobes dislodged and pushed up into the chest.

12:06. Respiration 10 per minute; lungs and blood dark.

12:08. Heart 102, respiration 6 per minute.

12:11. No pulse at femoral artery.

12:13. No respiration, heart beats very feeble. Attempt to redistend the lung by compressing the tube of the water valve.

12:15. No heart beat.

This animal with double pneumothorax lived under the Brauer method and remained in good condition for an hour and a half. *Effective stimulation of the vagi, cutting them, and stimulation of various parts of the pleura did not interfere with the favorable state.* But as soon as the lower lobes of the lungs were dislodged and pushed upward within the chest, the proper exchange of gases was profoundly interfered with, and the animal died fifteen minutes later.

The lower lobes in this case did not sink back into their normal place, although they were not fixed in their new position. The fixing of the dislodged lower lobes at a higher position within the closed thorax proved in two instances to be rather a favorable procedure.

Experiment 6.—Dog, weight 5 kilos. Brauer method; constant air pressure at 20 mm. of mercury and 15 cm. of water.

10:43. Both pleural cavities opened widely in the seventh intercostal space.

10:50. Heart 90, respiration 32, lungs well distended. Lower lobes freely detached, and by means of a strip of gauze carried through both openings between the lungs and diaphragm, the lower lobes were kept dislodged. In a few minutes respiration stopped completely and the heart became slow and feeble. The strip of gauze was then pulled upward tightly and secured so as to keep the lower lobes well fixed within the thorax above the opening. Respiration soon returned, somewhat labored, but apparently efficient; the heart beats strongly again.

11:20. Gauze relaxed; respiration stopped after two minutes; the heart stopped about a minute later. Various attempts at resuscitation (artificial respiration, insufflation, massage of the heart, adrenalin into the heart, etc.) failed.

The securing of the lower lobes within the upper part of the chest was apparently a life saving procedure, death following soon after the loosening of the supporting gauze strip.

We may mention that, as in this case, we tried in several other

experiments with the Brauer method to resuscitate the animals by means of insufflation or artificial respiration, etc., but we failed in every case.

With regard to our method of insufflation, besides the experiments which were made expressly in this series, we have observed on many other occasions that it is entirely indifferent to the success whether the lungs are within or without the thoracic cavity; the simple fact that curarized dogs are able to live for many hours under the insufflation method is an all-sufficient proof that the activity of any of the respiratory muscles can be dispensed with when using this method. However, for the sake of comparison and demonstration, we shall reproduce here a few protocols of the experiments made with the method of insufflation. This is the more justifiable, since these experiments will bring to light some other instructive points bearing upon our problem.

Experiment 7.—Dog, weight 9,700 grams. Insufflation method; constant air stream occasionally interrupted; pressure 25 to 35 mm. of mercury.

11:38. Both pleural cavities wide open.

12:13. Heart 123, respiration 42 per minute.

12:15. Insufflation interrupted for one minute; heart slowed strongly (but not to the same degree as in experiment 2 with the Brauer method, when interrupted only for 30 seconds).

12:21. Heart 90, respiration 24.

12:24. Insufflation stopped for one minute; heart slowed strongly.

12:38. Heart 102, respiration 36.

12:42. Insufflation stopped for 90 seconds; heart slowed very strongly towards end.

12:45. Heart 81, respiration 24.

12:49. Both lower lungs taken out of chest and held by clamps.

12:53. Heart 90, respiration 9.

1:10. Administered (by mistake) 6 mgr. of atropin.

1:15. Heart 72 per minute. *Started interruption of air stream, 20 per minute regularly.*

1:30. Heart 132, respiration 12 per minute; lungs pink.

1:35. Heart 124, respiration 20.

2:03. Stopped insufflation for 90 seconds; slight effect upon heart, did not stop respiration.

2:06. Heart 108, respiration 12 per minute. Animal killed by clamping aorta.

The animal lived about seventy-five minutes after removal of the lower lobes outside of the pleural cavities, and had to be killed; but the continuous insufflation was insufficient; regular intermissions

had to be introduced. A point of interest is the fact that a complete collapse of the lung had an incomparably smaller effect upon the activity of the heart than in any of the experiments with the Brauer method. *Even one hour after the removal of the lower lobes, a collapse of the lungs lasting ninety seconds had no great effect upon the heart or respiration.*

Experiment 8.—Dog, weight 14,500 grams. Insufflation with 20 remissions per minute; pressure 10 mm. of mercury.

11:55. Both sides wide open.

12:48. Heart 172, respiration 78.

12:50. Stopped insufflation for 2 minutes; no marked slowing of heart.

1:30. Heart 170, respiration 72. *Insufflation discontinued for 2 minutes; at the end, heart 154, respiration 66 per minute.*

1:55. Both lower lobes of lungs removed outside and fixed by clamps.

2:28. Heart 132, respiration 24 per minute. Respiration easy; condition of animal good. Killed.

Here again the removal of the lower lobes of the lungs from their position had no palpable effect upon the condition of the animal and again a prolonged collapse of the lung had a comparatively insignificant effect.

Experiment 9.—Dog, weight 12 kilos. Etherized; *stopped breathing.* Tube introduced into trachea, started insufflation with 20 remissions per minute; pressure, 25 mm. of mercury; chest massaged.

10:16. Animal breathes spontaneously; ether now given with insufflation.

10:25. Both pleural cavities opened widely in fifth intercostal space.

10:30. Smaller tube inserted into trachea.

12:05. Heart 172, respiration 18 per minute.

12:07. Stopped insufflation for 90 seconds; heart slowed and then stopped, respiration stopped. After insufflation started again, heart began to beat gradually; trachea compressed a number of times to distend lungs.

12:13. Heart 144, respiration 18 per minute.

12:15. Insufflation discontinued for one minute; heart slowed moderately, respiration did not stop.

12:16. Started insufflation; *heart more rapid at once.*

12:35. Both lower lobes outside of chest, held by clamps.

12:45. Heart 168, respiration 15 per minute.

1:45. Animal in good shape; fresh ether given.

2:34. Heart 153; animal in good condition. Killed.

This animal, under insufflation, remained in good condition for about two hours after the lower lobes of the lungs were completely dislocated and about four hours after both pleural cavities were widely opened, although at the beginning respiration and heart were

stopped by an overdose of ether. And here again the complete collapse of the lungs was far from exerting that deep influence which it exerts under the Brauer method, although in this experiment the influence was more pronounced than in the other two insufflation experiments.

In the protocols of the experiments with the Brauer method, reproduced in the foregoing pages, the increase of pressure within the lungs was accomplished by a constant air stream. That is the way over-pressure usually is maintained by the so-called Brauer method, and, indeed, various writers on this subject consider any variation in the pressure as a detrimental complication of the method. Unfavorable experiences, however, recently induced some surgeons,[8] in cases of failing respiration or circulation, to institute regular interruptions in the positive pressure which is to take the place of artificial respiration. We shall therefore reproduce here some abbreviated protocols of experiments in which the Brauer method was used with regular intermissions, not only under emergencies, but throughout the entire experiment.

Experiment 10.—Dog, weight 11,750 grams. Brauer method with 20 remissions per minute; 14 to 20 mm. of mercury pressure of air supply and 12 cm. for the water valve.

10:57. Both pleural cavities opened widely.

11:45. Heart 159, respiration 63 per minute.

12:04. Air supply discontinued for one minute; heart slowed only toward end of period; respiration quickened.

12:19. Air supply discontinued for 80 seconds: heart became extremely slow, respiration did not stop. After starting air stream again, *tube of water valve compressed once to redistend fully the collapsed lungs.*

12:23. Heart 150, respiration 48 per minute.

12:25. Lower lobes of lungs pulled out of chest and held by clamps.

12:30. Heart 80, slightly irregular, and respiration 15 per minute.

12:35. Heart 96, and respiration 9 per minute.

12:41. Heart 63 per minute; no respiration.

12:42. No femoral pulse.

12:45. No ventricular beats.

With 20 regular intermissions per minute of the positive pressure, the animal survived the dislocation of the lower lobes only by 16 to 18 minutes. The prolonged collapses of the lungs proved, however, in these experiments less pernicious than in the Brauer method without remissions; even an interruption of 80 seconds' duration did not stop the heart completely.

Experiment 11.—Dog, weight 14,750 grams. Brauer method, *continuous* pressure supplied by oxygen tank; pressure of water valve, 10 cm.

[8] W. Meyer, *Ann. Surg.*, 1910, lii, 53.

11:18. Both pleural cavities open.

11:48. Heart 80, respiration 27, slightly labored. Oxygen pressure slightly increased: respiration accelerated at once.

12:05. Heart 178, respiration 36; lungs pink.

12:06. Discontinued gas supply for one minute: heart slowed and dilated. Pressure increased to redistend lung.

12:11. Heart 96, respiration 20 per minute, labored.

12:15. Oxygen gave out. *Started continuous flow of air* with 20 remissions per minute.

12:18. Heart 172, respiration 42.

12:20. Air supply discontinued for 30 seconds: heart slowed powerfully, dilated.

12:35. Heart 144, respiration 48.

12:54. Positive pressure stopped for one minute; slowed swiftly to only a few beats, dilated. Started air stream: heart recovered swiftly.

12:58. Lower lobes of lungs taken outside of thorax and held with clamps.

1:04. Heart 84, very irregular; respiration 24.

1:11. Heart 30, respiration 6 per minute.

1:14. No respiration and no femoral pulse; heart beats feebly.

1:15. Heart stopped.

In the first part of this experiment, with a continuous oxygen stream, the respiration gradually slowed and became labored, apparently on account of the running down of the oxygen pressure in the tank. With the institution of the air stream with remissions, the respiration improved at once and for forty-three minutes the animal continued to be in good shape. As soon, however, as the lower lobes were securely dislodged from their position, the respiration rapidly slowed down until it stopped completely about sixteen minutes later.

Apparently the Brauer method, even with regular intermissions in the positive pressure, is incapable of maintaining the life of an animal with a double pneumothorax when the lower lobes of the lungs are completely dislodged.

We shall mention here one or two experimental observations which are of some interest but which did not come out with special sharpness in the protocols of the experiments reproduced here. In the first place, the pressure which keeps up a certain degree of distension of the lungs, is invariably insufficient to redistend the lungs to the same degree as before, after they are well collapsed. To accomplish this, a much higher pressure must be employed for a short time. The pressure must be higher the more complete the

collapse. This is true for the insufflation, as well as for the Brauer method.

In the insufflation method, the increase of the pressure is accomplished in a simple and convenient way by a short, lasting pressure upon the larynx. In the laboratory Brauer method, this can be accomplished by compressing the tube of the water valve. However, in the large chambers for differential pressure, employed in human surgery, the increase of pressure could be accomplished only by increasing the pressure outside of the chamber. Furthermore, in the insufflation method the interruptions employed are of very short duration and the lungs are only slightly reduced in volume. In surgery, however, where the interruptions in the positive pressure a few times a minute are instituted as a means of artificial respiration and the lungs are permitted to collapse completely, a distinctly higher pressure will have to be employed for the attaining of a satisfactory redistention. It does not seem to us that this indispensable procedure is employed in surgery; at least, we are not aware that any surgical writer speaks of it especially.

The knowledge of this fact is also indispensable in some experimental observations. For instance, in comparing the recuperative power of the heart after a prolonged interruption of the air stream, it is necessary to take into consideration the fact whether extra pressure was employed for the redistention.

The reason for this requirement is obvious. The original increase of pressure is instituted while the thorax is closed and the lungs are normally distended; the additional pressure is utilized entirely for bringing about an additional distension. After the lungs become collapsed, a certain degree of capillary adhesion is formed between the walls of some bronchioli and alveoli. The original pressure, therefore, is now insufficient to overcome two obstacles,—the capillary adhesion and the elasticity of the lungs.

DISCUSSION.

In our experiments, the openings in both pleural cavities were not excessive; but they were, nevertheless, definitely much larger than the lumen of the trachea and still more so than the intraglottideal space. Air entered, therefore, with much greater readiness through the artificial apertures into the pleural cavities than through the trachea even into inflated lungs. Furthermore, the lungs did not fill out the pleural cavities, so that even the lower lobes of the lungs did not come in contact with either the anterior walls of the chest or the anterior part of the diaphragm, especially when the openings were made in the seventh intercostal space. The movements of the mentioned parts of the thorax could, therefore, not

contribute to the exchange of gases within the lungs. Nevertheless, the exchange which actually took place was apparently sufficient to maintain the life of the animal for two hours or longer. On the other hand, it was constantly observed that life terminated promptly as soon as the posterior parts of the lower lobes of both lungs were completely dislodged from their position. The shortest time in which the animal died after the dislodgment was about three minutes; the longest time was about twenty-six minutes. The longer period occurred when the lungs repeatedly fell back into their normal position, or when the dislodgment was otherwise incomplete. It is a noteworthy point that after the dislodgment of the lower lobes death seemed to occur sooner, the longer the animal was exposed to the positive pressure with a double pneumothorax before the dislodgment took place. It is probable that there are many other factors capable of determining the length of time an animal survives the dislodgment of the lower lobes; for instance, perhaps the size of the openings, their locations in the intercostal spaces, the previous handling of the lungs, the degree of the positive pressure employed, etc.; but none of these factors was studied systematically enough by us to allow a positive opinion regarding their merits. On the other hand, we may readily admit that there might be conditions in which an animal, under differential pressure, may survive the complete dislodgment of the lower lobes even with longer periods than those observed by us; but in all probability the final fate will be postponed but not obviated.

The complete dislodgment of the lower lobe of one lung made, in the few cases we have studied, the respiration somewhat labored and definitely slowed, but in no case did this dislodgment lead to the death of the animal, at least not within the period during which the animal was observed by us; that is, for forty or fifty minutes after the dislocation of the lobe.

The detrimental effect of the dislodgment was chiefly upon the respiration which soon began to slow down until it completely stopped. The heart slowed down at first only moderately, and without exception the heart continued to beat after the complete stoppage of the respiration. Evidently the failure of the heart was in the main secondary to the respiratory failure.

The rapid respiratory failure could not have been due to reflex action by the way of the vagi, for the rapid fatal course was the same, whether the vagi were intact, or were eliminated by atropin, or cut half an hour before the dislodgment of the lower lobes took place. It is true that simple cutting of both vagi slows the respiration considerably, sometimes as low as ten or nine per minute. But the animal is able to live and to be in good shape for a long time with this slow respiration, the heart beats remaining unchanged and the color of the lungs and the blood being pink. Apparently the cause of death after the dislodgment of the lower lobes of the lungs in double pneumothorax under the Brauer method is asphyxia.

This fact has a practical and a theoretical significance. From a practical point of view, it is evident that in using differential pressure, positive or negative, it is dangerous to perform such operations upon intrathoracic organs which require a dislodgment of the lower lobes of the lungs. And even when both lobes are only partially dislodged, or when the lower lobe of only one lung is completely dislodged, there might be some danger of a gradual development of asphyxia, especially when in the process of the dislodgment even the adjacent lung is so handled as to drive out a great deal of the air of that part. Furthermore, the positive pressure in use will be insufficient, as stated before, to redistend properly the atelectatic parts and place them in proper position. The course of the fatal asphyxia in the last mentioned conditions need not, as indicated before, be a rapid one; the patient might even come off the table in a seemingly tolerable state with a slow respiration and a tolerable circulation. The insufficiency of the exchange of gases may gradually lead to a quiet fatal termination of both functions.[9]

As to the theoretical value of our results, the experiments show definitely that the maintenance of the life of the animal under differential pressure depends upon the proper position of the posterior part of the lower lobes of both lungs. Even if we assume that these

[9] See, for instance, W. Meyer, *Ann. Surg.*, 1910, lii, 34. Case ii, operation on right inferior lower lobe; patient died on operating table. Case iii, operation on left lower lobe; patient died about one hour after operation. Case iv, carcinoma of esophagus, lower end of tumor about one inch above cardia; patient died about ten minutes after finishing dressing.

parts distend and retract in a widely open thorax to the same extent that they do in a closed one, these parts form only a comparatively small fraction of the entire lungs. The experiments teach us, in the first place, that the life of the animal may continue, seemingly in a perfectly satisfactory condition, under a respiration which presents only a small fraction of the normal respiration. We may recall here the fact that the complete dislocation of the lower lobe of only one lung does not yet terminate the life of the animal, at least not for a time. Accordingly, the requirement of exchange of gases for maintenance of life is indeed very small compared with that which actually takes place in normal conditions.

We now return to the original puzzling question, How can the exchange of gases, necessary for keeping up life, be accomplished in the presence of openings in the thorax which are much larger than the lumen of the trachea? We answer, in the first place, that our conception of the degree of respiration which is absolutely necessary for the maintenance of life is based upon the observation of the normal respiration, and is thoroughly erroneous. The normal respiration shows the function in a luxurious state, when it is provided with an abundance of factors of safety. The actual necessity of respiratory function for the mere maintenance of life amounts only to a small fraction of the normal respiration; and this small fraction is present even in a double pneumothorax, when the lungs are distended by extra pressure, positive or negative.

The following considerations will show us some essential points of the advantages of the differential pressure. When the lungs are subjected to the atmospheric pressure in a double pneumothorax without the neutralizing action of a differential pressure, most of the air is driven out, and the walls of a good many of the alveoli and the bronchioli become conglutinated by capillary adhesion. In the presence of wide openings of the thorax, the fraction of air pressure to which the lumen of the trachea is entitled during an inspiratory distention of the thorax, is insufficient to overcome the combined resistance of the capillary adhesion of the alveoli and the elasticity of the lung tissue, and no exchange of gases can take place within the lungs; the result is therefore a rapid suffocation. The proper position of the lower lobes of the lungs is under these circumstances of no advantage. Under the influence of a proper differential pressure, positive or negative, the alveoli and bronchioli remain open even if the entire outer surface of the lung is exposed to atmospheric pressure, and capillary adhesion is not a disturbing factor in the mechanism of the crippled respiration. This prevention of the complete collapse of the lungs

with the consequent detrimental factor of capillary adhesion is indeed the chief service which the Sauerbruch and the Brauer methods render to the respiration in the face of an open pneumothorax. But the differential pressure has no share in the respiration itself; this has to be accomplished by the respiratory movements of the animal. These movements, however, render no service to the proper exchange of gases, if the atmospheric air has an unrestricted access to the entire outer surface of the lungs; the quota of air which, under these circumstances, may enter and leave the lungs by way of the trachea, is by far too small to support the processes of life. Our experiments have shown that at least the posterior part of the lower lobe of one lung must remain in position and be free from the direct access of air to the outer surface, if the exchange of air should be sufficient to keep up life.

If we leave for the present the discussion of the manner in which these parts assist in the exchange of gases, it is evident that this rudimentary respiration presents, as stated before, only a small fraction of the exchange of gases which takes place in the normal respiration with closed thoracic cavities. In discussing the factors of safety with which the lungs are provided, it was said: "We have here, with regard to the quantity of tissue, a factor of safety equal at least to two, which does not appear an excessive margin considering the importance of the function which that tissue has to carry out."[10] Our experiments show that the important function of the exchange of gases is provided indeed with a great abundance of factors of safety. Indeed, Sauerbruch,[11] who removed large parts of the thorax without finding that the normal course of the respiration of the animal placed in his cabinet was seriously affected by it, came to the conclusion that "das Atmungsbedürfniss ein sehr geringes ist." He states that the first appearance of dyspnea occurs when the respiratory volume is reduced to one tenth or one half of the normal. Without laying stress upon definite figures, we believe that, with regard to the abundance of factors of safety, the respiratory functions of the lungs may be readily compared with the glycolytic function of the pancreas, of which gland we know that the retention of one tenth is sufficient to prevent the acute onset of diabetes.

In the gradual reduction of the thoracic cavity, Sauerbruch removed consecutively various parts of the thorax without pointing

[10] Meltzer, *Jour. Am. Med. Assn.*, 1907, xlviii, 655.
[11] Sauerbruch, *loc. cit.*

out especially which residual part of the thorax is the most essential for the maintenance of the respiratory function. In our experiments, we practically did not destroy any part of the thorax; we only made wide openings in the intercostal spaces, one on each side of the thorax. Nevertheless, simple dislodgment of the posterior parts of each lower lobe was sufficient to cause the death of the animal in a short time, and this often when the positive pressure kept the upper lobes so well distended as to fill out the upper parts of the intact thorax. Only when the openings were made in the seventh intercostal space and the lower lobes which were separated from the diaphragm were tightly pressed upward by means of a gauze strip, could the respiration be maintained for some time. We have, therefore, sufficient reason for the claim that *under differential pressure it is essentially the close approximation of the posterior part of the lower lobes to their normal surrounding which keeps up the indispensable minimum of respiration.*

As to the mechanism by which the fraction of respiration is accomplished, Sauerbruch describes it, for the case in which the sternum and the ribs are removed, as follows: By the expiratory movement of the diaphragm headwards, the lungs become slightly compressed and some air is driven out, which partly escapes outside through the trachea and partly enters into the free parts of the distended lungs. During the inspiratory contraction of the diaphragm, the lower parts of the lungs, becoming relieved from the expiratory pressure, redistend, and thus aspire air again. Sauerbruch apparently assumes that the expiration in these cases is an active process, presumably by the aid of the contraction of the abdominal muscles. We have had, however, experiments with the Brauer method in which the cord was cut at about the sixth cervical vertebra. Here the abdominal muscles were eliminated, and the respiratory muscles of the thorax could not contribute much to the respiration; nevertheless, the animal continued to live in the same satisfactory condition as an animal with an intact cord. On the basis of our experiments, we are inclined to assume that the respiration is carried on essentially by the inspiratory action of the diaphragm. As long as the lower lobes of the lungs are in a normal position and closely adapted to the diaphragm, the con-

traction of the posterior part of this chief inspiratory muscle is capable of producing an efficient inspiratory distension in the adjacent part of the lungs. The negative pressure created in the lower and posterior part of the thorax by the contraction of the posterior part of the diaphragm, can become compensated more readily by the entrance of air into the adjacent parts of the lungs than by the pressure exerted upon the outer surface of the anterior parts of the lungs exposed directly to the atmospheric pressure. The alveoli and bronchioli of the lower and posterior parts of the lungs are kept open by means of the positive pressure and are thus in direct communication with the atmospheric air, while they are separated from the atmospheric pressure resting upon the outer surface of the exposed lung by innumerable elastic air cells.

On the basis of our experiments and of those of Sauerbruch, we arrived at the conclusion that respiration in animals with mutilated chests under differential pressure presents a very small fraction of the extent of the respiration which takes place with unopened chest cavities. In the experiments of Sauerbruch, the thorax of the animal was removed in a manner which would never occur in human surgery. In our experiments, however, the thorax was opened only in the intercostal spaces and was surely not more mutilated than in any surgical operation. It was shown, nevertheless, that life was sustained only by the normal position of the lower lobes of the lungs, which means, as shown, that life in these conditions is sustained only by a small fraction of the normal exchange of gases. Now these facts bring into prominence another aspect of the differential pressure methods which is of considerable importance. *During an intrathoracic operation under differential pressure, the function of respiration of the operated individual is, to say the least, deprived of all or most of its factors of safety.* Let us consider what this might signify. In a large number of instances, it was shown[12] that the mechanisms of the animal organism are built on the same plan as human machines; that is, with a provision of a large number of factors of safety. For the absolute safe working, a structure or a machine must be constructed on the following plan: it must (1) be able to accomplish the work neces-

[12] Meltzer, *Jour. Am. Med. Assn., loc. cit.*

sary to meet the average demand; it must (2) also be in absolute readiness to meet the exceptional demands now and then occurring; and to both (3) an extra safeguard must be added to be in readiness to meet entirely unforeseen exigencies. This third addition is termed in engineering "factors of safety." The extent of this last addition varies with the design for which the machine or structure is built. We need not dwell here on the details of this problem. In our concrete subject, it is evident that the small fraction of respiration present in an open thorax under differential pressure is surely bare of any factors of safety. Furthermore, it is highly probable that it would fail to meet even the expected exceptional failures like shock, fall in blood-pressure, irregularities of respiration and the heart. In our experiments, we met with facts which indicated that the respiration under positive pressure, which gave the appearance of keeping up life satisfactorily, was actually undermining the efficiency of the two most important factors of life,—the respiratory center and the heart. In the protocols of the experiments reproduced above, instances will be found in which, when the positive pressure was interrupted for only a very short time there was immediately a strong falling off in the activities of the heart and the respiration, while in the experiments under the insufflation method, such an intense effect was not brought about even by interruptions lasting for a longer period of time. Furthermore, in the Brauer method, the recovery of these functions after the restoration of the positive pressure was usually very slow. Finally, if, after the dislocation of the lower lobes, the respiration stopped and the heart became feeble, the institution of insufflation or of efficient artificial respiration failed to restore life, especially when the animal was previously for a long interval under the Brauer method; while after the interruption of the insufflation method, the animals may be often readily revived even minutes after the heart stood still. All these facts go to show that during the respiration under the over-pressure method, while the animal conveys the impression of being in a normal state, the center of respiration as well as the heart muscle fibers lose apparently a great deal of their efficiency, so that a comparatively unimportant incident interferes greatly with their activity and might even terminate completely their vitality.

In other words, the function of respiration under differential pressure is deprived of all factors of safety, and is, in all probability, even incapable of meeting exceptional emergencies; the power of resistance, as well as the recuperating power of the respiratory center and of the cardiac mechanism during respiration under differential pressure are greatly undermined. In short, the life of the individual with an open pneumothorax under differential pressure is exposed to a variety of grave dangers.

Several cases of deaths were reported as occurring while under differential pressure or right after it. They were explained as having been caused by shock or some vagus reflexes. We shall not discuss shock. But that death is produced by vagus reflexes, is, to say the least, as yet an unestablished fact. In our experiments with the Brauer method, we have, at various times, stimulated the intact vagi, or the ends of the cut vagi, with electric currents strong enough to stop the heart beats and also the respiration. In no case have we observed any kind of a permanent detrimental effect from it. Neither did we observe any kind of an effect worth speaking of from stimulation of any part of the parietal pleura. Cutting of both vagi brought on an acceleration of the heart beats and a slowing of the respiration.[13] But the animals continued to live for some time after the cutting, apparently in the same safe state as they were before. For the present, we have every reason to assume that the deaths occurring under differential pressure, if they were not brought about by the dislodgment of the lower lobes of the lungs, were caused, in the last instance, by the low state of the function of respiration, which for some time has been deprived of all safeguards, so that the slightest incident could bring on an irrevocably fatal effect.

Turning now to our experiments made with intratracheal insufflation, we can be very brief. In all the experiments, the complete dislodgment of the lower lobes of the lungs had not the slightest detrimental effect upon the further course of the life of the animal;

[13] With regard to our observations upon the stimulation of the vagi and pleural cavity, we have to recall the fact that in all our experiments the animals were under ether anesthesia. But so were the patients in which sudden deaths occurred.

they lived as long as they were permitted to live,—for an hour or two, or longer. And even with the lower lobes of the lungs outside of the thoracic cavity, when the insufflation was interrupted for a minute or two and the lungs completely collapsed, the respiration and heart did not stop readily, and when they did stop, the resumption of insufflation usually restored the heart and respiration fairly rapidly. We need not be surprised at that, since even completely curarized animals will continue to live under the method of intratracheal insufflation. It may perhaps be profitable to point out the extent of the factors of safety with which this method is surrounded. The respiratory surface in the alveoli is separated from the air by the long shaft composed of the mouth, pharynx, larynx, trachea, and bronchial tree. The entire neuro-muscular respiratory apparatus is created for the purpose of causing ventilation through this long, winding air shaft. In the method of intratracheal insufflation, air is forcibly brought down to the lower end of the trachea and is driven out by the same force. The longest part of the air shaft is in this way cut out, and there remains only the bronchial tree which separates the respiratory surface from the fresh air at the bottom of the trachea. It was shown by experiment[14] that, when the insufflation tube within the trachea is in proper proportion to the lumen of the trachea, the continuous insufflation is sufficient to keep up the life of curarized animals for many hours. This implies that the driving of air forcibly to the end of the trachea affects also the air within the bronchi so as to facilitate there a mixing and diffusion of the gases to an extent which, under certain conditions, is sufficient to maintain life without the aid of muscular movements of the thorax. In the non-curarized animals, with which we deal here, the respirations of the animal itself are added to the insufflation. The extent of this respiration is at least equal to that occurring in the Brauer method, but it must be remembered at the same time that in the Brauer method the fresh air is to be obtained only at the mouth of the individual (in the chamber or mask), while in the insufflation method the fresh air is met with already at the end of the trachea. Finally, in the insufflation method in the practical form in which it is now used, the continuous

[14] Meltzer and Auer, *Jour. Exper. Med.*, 1909, xi, 622.

stream is interrupted several times per minute. The interruptions are of short duration; but during each interruption the lungs collapse slightly and drive out the carbon dioxid-laden air from the bronchi into the domain of the trachea; and at the instant of the resumption of the insufflation, fresh air is driven directly down into the bronchi. These remissions are all sufficient to ventilate completely the bronchial tree. With an interrupted insufflation we are capable of keeping up the life of thoroughly curarized animals for many hours in a very satisfactory state, and with a stable, good blood-pressure.

In intratracheal insufflation, then, we have for the maintenance of the respiratory function: (1) the insufflation which cuts out the dead space of the ventilation shaft to the end of the trachea and causes some degree of forcible diffusion of gases within the bronchial tree; (2) the short interruptions of the insufflation stream, a procedure which acts as a sort of artificial respiration and is sufficient to ventilate completely the bronchial tree; and (3) the respirations of the animal itself. All three processes together surround the function of respiration with an abundance of factors of safety equal at least to those of normal respiration. In contrast to this stands the differential pressure relying alone upon the respiration of the animal *itself, which under the given circumstances amounts only to a small fraction of the normal respiration.*

In conclusion, we repeat that, although our experiments were made with the positive pressure method alone, our results apply with equal force to the negative pressure method, as there is practically no difference between the two methods, at least, as far as the points brought out in this paper are concerned. We have therefore often spoken in this paper interchangeably of the Brauer method and the methods of differential pressure.

SUMMARY.

The maintenance of life of an individual with an open double pneumothorax under differential pressure depends essentially upon the normal position of the lower lobes of the lungs and their close approximation to the diaphragm, especially of the posterior parts of the lobes. A complete dislodgment of both lower lobes leads invar-

iably to the death of the individual, which may occur in a very short time, or after fifteen to twenty-five minutes. In all cases the respiration is affected first; it slows almost at once and stops invariably before the heart. The result is the same whether the vagi are intact or both nerves are cut. Exceptionally, respiration may continue even after the separation of the lungs from the diaphragm, but only by having all the lobes well approximated to the thoracic walls. When by dislodgment of the lower lobes the respiration is stopped and the heart is feeble and slow, or stopped completely, it is rarely possible to restore life by artificial respiration or by other appropriate means.

The extent of the exchange of gases occurring in normal respiration, with closed thoracic cavity, exceeds greatly the need for the maintenance of life, since normal respiration is provided with an abundance of factors of safety. Under differential pressure, however, life is carried on with an exchange of gases which amounts to a small fraction only of the extent of the exchange that takes place in normal respiration; respiration under differential pressure is, therefore, deprived of all factors of safety and is incapable of resisting the dangers of exceptional incidents. Deaths occurring in connection with the differential pressure have their cause essentially in this unguarded state of the function of respiration.

Under tracheal insufflation, the function of respiration is surrounded with effective safeguards, at least as much as is normal respiration. Dislodgment of the lungs has no detrimental effect.

After complete collapse of the lungs, capillary adhesions within the alveoli and the small bronchi become an additional obstacle to the redistension and respiration. Differential pressure holds this obstacle in abeyance.

When the lungs become collapsed during an open pneumothorax, it should be kept in mind that the force which is required for redistension is greater than that which is sufficient to keep the lungs continually distended. At the beginning of a redistension, therefore, a higher pressure should be employed for a short time.

STUDIES ON THE VENOUS PULSE.

II. The Time Relations of the Venous Pulse and the Heart Sounds.*

By J. A. E. EYSTER.

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Plate 53.

The present communication embodies the results obtained from a study of venous pulse, heart sound, and cardiographic records obtained from seventeen men with normal hearts, and from venous pulse, heart sound, and carotid records from the dog.

Four series of data are summarized in table I, as follows: (*a*) average of measurements of different features relating to the venous pulse and the heart sounds in fifty cycles from sixteen individuals; (*b*) similar data from thirty-six cycles in one individual; (*c*) average of measurements in cardiac cycles of approximately the same length in thirteen individuals; (*d*) averages showing the effect of increase in pulse rate from exercise in one individual. The observations included in the first and third series were made upon subjects under conditions as nearly as possible the same as regards quietness of surroundings and previous activity. The subjects were medical students in good health and with normal hearts. The observations included in the second series were made at different times extending over a period of about six months. The subject was a healthy, vigorous man, a laboratory assistant, aged 31 years, with a normal heart. The same subject served for the observations upon the effect produced by increase of pulse rate from exercise.

The main objects in view in this work were three: (1) To obtain the average position of the first, second, and third heart sounds on

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the normal venous pulse, and the variations from this average position. (2) To determine which waves and which intervals on the venous pulse tend to preserve a fixed position and which are most variable. The first and second heart sounds were chosen as representing definite occurrences in the cardiac cycle and as being constant in position in their relation to these occurrences. (3) To determine which features of the venous pulse and of the heart sound intervals tend to remain constant in duration with variations in the length of the cardiac cycle, and which show a tendency to be proportional to the length of the cycle.

Methods.—The heart sounds were recorded by the method of Einthoven (1). A funnel, placed over the apex region of the heart, was connected by means of a rubber tube to a microphone. A lateral branch of this tube near the funnel was left wide open in communication with the outer air to prevent the larger and slower movements of the chest wall, caused by the apex beat, from affecting the microphone. The microphone was in the primary circuit of a small induction coil. The secondary circuit of this coil was connected with an Einthoven thread galvanometer of the small electro-magnet type (Edelmann model). By means of an arc lamp and a series of suitable condensing lenses, the thread of the galvanometer was projected on a photographic registration apparatus and the movements of the thread produced by the heart sounds were photographed on bromide paper or films. A delicate recording tambour connected with a Mackenzie venous pulse transmitter and a tuning fork vibrating 100 times per second, or a Jaquet chronograph marking one fifth second intervals, were also arranged to cast shadows on the slit of the photographic registration apparatus. The registration apparatus was provided with an arrangement for producing coördinates on the record, and these greatly facilitated the determination of incidence of the various features on the record and of measurement of small differences in time.

In most records, the speed of the apparatus was so regulated that one hundredth of a second was represented by a space of from one to two millimeters (figures 1 and 2). The latency of the thread of the galvanometer to single induction shocks and the latency of the venous pulse transmitting and recording apparatus

were compared. The difference in the latency of the two was found to be less than 0.002 of a second, and no corrections were made in the tables.

Observations were all made with the subjects in the recumbent posture. In all cases, immediately following the completion of the venous pulse and heart sound records, and without allowing the subject to move or change his position, a cardiogram and venous pulse record was made on blackened paper for the purpose of determining transmission time and for comparison of the position of the cardiogram.

In the dog, the heart sounds were recorded as described above. The venous pulse tracings were obtained by the use of the venous pulse sounds devised by Fredericq (2), which were introduced into the external jugular vein and passed down into the right auricle. The fenestrated end of the sound, before introduction into the auricle, was covered with a section of everted vein, with its endothelial surface outward. This procedure makes it possible to leave the sound for long periods of time in the auricle without the formation of clots. The sound was connected by a rubber tube with the same recording tambour used in man.

Explanation of the Tables.—In table I are given averages and variations from full tables containing the measurements from the venous pulse and heart sound records. The figures represent hundredths of a second, except in the percentage columns. For example, 19.2 is to be read 0.192 of a second. In the first two series of data, besides the averages, total variations are given. These figures represent the differences between the greatest and smallest value occurring in the full table from which the averages were obtained. If we take, for example, the *a–c* interval in the first series, the average length from all values in the full table was found to be 0.192 of a second, while the shortest *a–c* interval in any of these cycles was 0.125, and the longest 0.27 of a second, making a total variation of 0.27–0.125 or 0.145 of a second. The percentage variation of the *a–c* interval in the same series, 22.7, does not refer to the actual average variation, but likewise is the difference between the highest and lowest percentage occurring in the full tables. These percentages were obtained, in other words, not from the average length of the cardiac cycle given in table 1, but from the length of the particular cardiac cycle in which the measurement was made. Finally, it is to be noted that there is not, in all cases, a balance between the figures in the tables, due to the fact that in the full tables a few measurements were omitted in the case of certain cycles, because for one reason or another accurate measurement could not be made.

The length of the cardiac cycle was determined by the time interval from the beginning of the first heart sound to the beginning of the first sound of the next cycle.

Subject.	Character of data.	Cycle.		Intervals on venous pulse.						
		Length.	Character.	*a–c*	*c–v*	*v–h*	*h–x*	*v–a*	*h–a*	*x–a*
Sixteen individuals with normal hearts	Average of 50 cycles.	85.4		19.2	29.4	20.7	12.7	37.1	20.9	15.0
	Total variation from averages	41.0		14.5	13.0	13.0	6.0	40.0	32.0	15.5
One individual with normal heart	Average of 36 cycles	87.9		17.0	28.8	16.2	16.7	39.1	24.9	17.9
	Total variation	35.0		9.0	15.0	6.0	6.0	28.0	35.0	16.5
Thirteen individuals with normal hearts	Averages of cardiac cycles of approximately the same length from different individuals	77.4	No *h*-wave	17.7	28.2	22.3		30.6	14.7	
		91.0	*h–x*	21.5	28.2	18.5	14.3	43.9	26.4	14.7
		102.7		16.8	30.5	20.5	14.6	53.5	33.0	21.0
Effect of exercise in one individual	Average of 3 cycles before exercise	88.3	*h*-wave	17.3	31.7	13.5		38.7	23.8	
	Average of 3 cycles after exercise	68.5	No *h*-wave	21.5	26.0			18.0		

Length of cycle.	Cardiogram.	
	Length of ascending limb.	Length of plate
91.9	8.8	22.4

TABLE I.

venous pulse.				Intervals on venous pulse. Percentage of cycle.								Transmission time.	First sound.					Second sound.				
														Relation to venous pulse. Uncorrected.		Relation to venous pulse. Corrected.			Relation to venous pu Uncorrected.			
v–*a*	*h*–*a*	*x*–*a*	Length of *c*-wave.	*a*–*c*	*c*–*v*	*v*–*h*	*h*–*x*	*v*–*a*	*h*–*a*	*x*–*a*	Length of *c*-wave.		Duration.	Before *c*.	Before crest of *c*.	Before *c*.	Before *v*.	Duration.	Begins after first sound.	After *c*.	After crest of *c*.	Before *v*.	Before crest of *a*
37.1	20.9	15.0	16.4	22.6	34.6	23.5	13.2	42.3	22.8	15.6	19.5	6.6	12.8	9.7	16.7	3.1	33.0	10.1	32.8	23.6	16.4	5.7	15.9
40.0	32.0	15.5	3.5	22.7	20.0	18.9	6.0	25.6	24.3	6.5	8.7	6.0	9.5	6.0	7.0	7.0	14.0	7.0	12.0	11.0	11.0	13.0	12.0
39.1	24.9	17.9	16.0	19.6	34.0	19.2	17.0	45.1	27.3	18.2	18.7	8.0	12.7	11.3	17.9	3.3	32.5	8.9	36.4	25.2	19.3	4.5	11.9
28.0	35.0	16.5	5.0	13.7	18.0	6.5	9.1	21.7	27.3	14.4	9.0		6.0	8.0	8.0	8.0	13.0	5.0	9.5	11.0	8.0	10.0	11.5
30.6	14.7		16.8	23.5	36.0	28.0		39.0	18.5		20.6	6.8	11.3	9.2	17.0	2.4	33.3	9.2	32.0	23.0	15.0	6.5	15.3
43.9	26.4	14.7	16.0	23.6	30.9	20.5	15.7	49.5	28.8	16.6	16.8	7.2	14.1	11.0	16.2	3.8	32.4	9.9	34.7	22.9	17.4	5.4	15.2
53.5	33.0	21.0	17.3	16.2	28.3	19.8	14.0	51.6	31.3	20.1	16.7	6.6	14.2	11.1	18.0	4.5	34.0	11.0	36.7	26.5	18.8	4.9	15.1
38.7	23.8		19.5	19.6	35.8	15.3		43.6	26.9		22.0	8.0	11.8	11.1	15.0	3.1	35.5	11.6	33.0	21.3	17.0	10.5	15.7
18.0			17.5	31.1	37.7			26.4			25.6		10.8	8.3	13.2	0.3	26.0	7.7	28.5	19.3	14.2	8.5	16.2

TABLE II.

Cardiogram.			Relation of cardiogram to venous pulse. Corrected for transmission.				Relation of cardiogram and for tran	
d-	Length of plateau.	Length of descending limb.	*v* begins before end of plateau.	*v* begins before foot of descending limb.	Foot of descending limb precedes *h*-wave.	Foot of descending limb occurs after crest of *v*-wave.	Foot of ascending limb of cardiogram and foot of ascending limb of carotid pulse.	Apex its b
	22.4	10.3	0.3	11.3	16.1	1.0	7.8	

	Third sound.										Heart sound intervals.						Systole.		Diastole.	
	Relation to venous pulse. Corrected.		Duration.	After 1st sound.	After 2d sound.	Relation to venous pulse. Uncorrected.			Relation to venous pulse. Corrected.		1st–2d.		2d–3d.		3d–1st.		Actual.	Per cent. of cycle.	Actual.	Per cent. of cycle.
Before *h*.	Before or after *v*.	Before crest of *v*.				After *v*.	After crest of *v*.	Before *h*.	After *v*.	Before or after *h*.	Actual.	Per cent. of cycle.	Actual.	Per cent. of cycle.	Actual.	Per cent. of cycle.				
26.0	+1.0	9.3	3.3	51.1	17.2	11.5	1.5	8.0	18.1	−1.4	32.8	39.4	17.2	20.5	34.0	40.0	30.7	35.0	55.7	64.2
9.5	15.0	16.0	8.0	16.0	5.0	10.0	8.5	6.5	12.0	9.0	13.0	15.1	5.0	12.4	31.0	23.9	7.5	14.0	28.5	14.0
20.3	+3.5	3.9	3.9	52.3	15.8	11.0	3.7	4.7	19.0	−3.3	36.4	41.9	15.8	18.2	35.8	39.9	33.1	37.6	55.0	62.4
9.0	10.0	11.5	4.0	10.5	4.0	9.5	10.0	8.0	9.5	8.0	9.5	13.5	4.0	10.8	31.0	19.4	11.0	14.8	27.0	15.3
26.2	+0.3	8.5	4.0	50.9	17.3	12.0	2.0	9.0	18.8	−2.2	32.2	41.0	17.3	21.9	29.3	36.9	29.9	38.4	48.0	62.0
24.1	+1.8	8.0	2.4	51.5	16.9	11.8	2.5	6.2	19.0	+1.0	34.7	38.2	16.9	18.2	39.4	42.8	30.6	33.6	60.2	66.4
25.5	+1.7	8.5	4.9	53.0	15.9	11.6	3.2	9.0	18.2	−2.4	36.7	35.7	15.9	15.2	50.1	48.8	32.2	31.2	70.6	68.8
24.0	−2.5	7.7	4.7	50.0	16.8	7.0	1.3	7.6	15.0	+0.4	33.0	37.4	16.8	19.0	38.5	43.6	29.5	33.4	58.8	66.6
	−0.5	8.2	6.5	40.7	12.2	4.5	−3.5		12.5		28.5	41.5	12.2	18.1	27.7	40.4	26.6	38.7	41.5	61.3

arotid pulse. Uncorrected nission.		
pulse after ginning.	Deepest part of dicrotic notch after end of plateau.	Transmission time.
.8	4.8	6.1

In most venous pulse records, the *c*-wave ends a considerable period of time (0.10 of a second, or more) before the *v*-wave begins, and between the two is a positive wave, which in some cases may be due to a fling of the recording lever, but in others is evidently a definite feature of the cardiac cycle. This wave in the nomenclature proposed by Bard (3) is designated as the *t*-wave, while the *v*-wave of English and American authors is designated as *d*. In certain cases, the *t*-wave may be a very small wave at the foot of the descending limb of the *c*-wave, or, as described by Bard, it may occur later and the separation from the *v*- (or *d*-) wave may be indicated only by a notch. In still other cases, it occurs on the descending limb of the *c*-wave (figure 2). In view of the uncertainty of the occurrence and character of this wave, it was thought best not to include it in the tables, but to measure the length of the *c*-wave and the *c–v* interval. The length of the *c*-wave includes the time from the beginning of the wave to the end of the descending limb. It includes, therefore, the positive *c*-wave and a part of the negative phase, usually designated by the letter x.

The measurements of the relation of the venous pulse waves to the heart sounds refer in all cases to the beginning of the sound and the beginning or crest of the wave, as stated. The relations noted as uncorrected in the table have not been corrected for transmission time. The plus sign before any figure indicates an occurrence after the feature noted, while a minus sign indicates its occurrence before the feature.

Systole was measured as the interval between the beginning of the *c*-wave and the beginning of the second heart sound, plus the period of transmission of the venous pulse wave from the heart to the great veins of the neck. Since transmission time was determined as the difference between the foot of the ascending limb of the cardiogram and the *c*-wave on the venous pulse, systole represents, therefore, in these measurements, the time from the beginning of the apex beat to the second heart sound. The reasons for accepting this interval as the duration of systole will be discussed later.

Table II consists of averages from measurements of cardiographic and venous pulse, and carotid and venous pulse records, obtained from the same subjects who served as the source of data in table I. These records were made to obtain transmission time in the individual cases and to furnish data for the plotting of the cardiogram and carotid curves in the diagram of the cardiac cycle (figure 5).

RESULTS.

When corrected for transmission, the first heart sound begins, on an average, 0.031 of a second before the *c*-wave, but it may begin as early as 0.07 of a second before, or it may be coincident with it. The second heart sounds begins, in the majority of cases, when correction is made for transmission, a few hundredths of a second after the beginning of the *v*-wave. Its occurrence has been observed as early as 0.045 of a second before, and as late as 0.15 of a second after the beginning of the *v*-wave. The *v*-wave is

therefore usually, but not always, a diastolic event, since the first part may be included in late systole. The *v*-wave shows a greater variation in its relation to the first and second sounds than does the *c*-wave to these sounds, or the *h*-wave to the third sound. It would seem to be the most inconstant in position of any of the various pulse waves. The third sound shows a fairly constant relation to the beginning of the *h*-wave, preceding the beginning of this wave in the majority of cases by a few hundredths of a second.[1]

Of the various pulse intervals, those that show the least variation in different cycles are the *a-c, c-v, v-h,* and *h-x* intervals.[2] The *v-a, h-a,* and *x-a* intervals, on the other hand, show marked variations. In percentage of the cardiac cycle, the greatest variation is shown by the *v-a* and *h-a* intervals, which is, however, less than the actual variation. On the other hand, the *a-c, c-v,* and *v-h* intervals, and the length of the *c*-wave, vary more in percentage of the total cycle than they do in actual measurement. The significance of this difference will be discussed later.

The first heart sound has an average duration of 0.128 of a second. The shortest recorded was 0.065, the longest 0.16 of a second. It is somewhat longer in longer cardiac cycles, largely due to its earlier occurrence before the *c*-wave. The second heart sound averages about 0.095 of a second. The shortest recorded was 0.07, the longest 0.13. It likewise tends to be of greater duration the longer the cardiac cycle.

The third sound averages 0.036 of a second with a total variation between 0.02 and 0.10 of a second. The maximum length reported occurred in only one case and is unusual; the longest noted in any other individual was 0.06 of a second. The records do not indicate that it increases in length with increase in length of the cardiac cycle, as is the case with the other heart sounds.

Table I gives, in the case of the venous pulse intervals, the heart sound intervals and the relation of systole and diastole, per-

[1] For literature on the third sound and *h*-wave, consult Hirschfelder (4), Einthoven (5), Gibson (6), Thayer (7), and Eyster (8). The association between the *h*-wave and the third sound does not seem to be as noteworthy as might be assumed from the above statement. Our records show that either one may occur and be well developed in the absence of the other.

[2] For a description of the *x*-wave see Eyster (8).

centage of the total cycle, as well as actual lengths, and the total variation of both percentage and actual measurements. These measurements were made in an attempt to determine, by the data at hand, which of the features of the cardiac cycle tended to remain constant and more or less independent of the length of the cardiac cycle, and which of the features were most affected by the total length of the cycle. It will be seen from the table that in certain cases there is a greater actual, than percentage variation, while in other cases the reverse is true. It will be well to discuss briefly the meaning of this in relation to the light it throws on the data given above.

Any feature which is strictly proportional to the total length of the cardiac cycle shows no percentage variation, but shows actual variation when cycles of different lengths are considered. When, therefore, percentage variation is smaller than actual variation, there is a tendency to vary with the length of the cycle and to be proportional to it. On the other hand, when percentage variation is greater than actual variation, the length of the feature tends to be a fixed one and independent, to a greater or less degree, of the length of the cycle.

If we examine the figures given for the actual and percentage variations of the venous pulse intervals, the heart sound intervals, and systole and diastole in the first two series of data of the table, we may form two groups, as follows:

Group I.	Group II.
Percentage variation smaller than actual variation	Percentage variation greater than actual variation
Venous pulse intervals	Venous pulse intervals
v–a	*a–c*
h–a	*c–v*
x–a	*v–h*
Heart sound interval	Heart sound interval
3d–1st	1st–2d
Diastole	2d–3d
	Systole

The features presented in group I, therefore, tend to vary with and to be proportional to the total length of the cycle, while those in group II tend to be more constant in length and more independent of the total length of the cardiac cycle.

In a consideration of the data presented in the third series of figures, those from thirteen individuals in which cardiac cycles of approximately the same length are averaged and compared with those of different duration, we may apply a similar method of reasoning. If we consider first systole and diastole, we find that the actual length of systole here increases with increase in length of the cardiac cycle, while the length of systole considered as a percentage of the cycle decreases as the cycle becomes longer. Furthermore, the percentage change is greater than the actual change. Both of these facts indicate the tendency of systole to remain constant and to a considerable extent independent of the length of the cycle. In the case of diastole, there is both an actual and percentage increase with increase in the length of the cycle. The percentage increase is much smaller than the actual increase. Both of these facts seem to show the tendency to variation in length of diastole with changes in length of the cardiac cycle. Similar considerations applied to the other features of the third series of data will result in the formation of two groups, as given above.

From this series of data we find, furthermore, that with an increase in the length of the cardiac cycle there is: (1) the appearance of the *h*- and *x*-waves; (2) no noteworthy change in the actual duration of the *a-c, c-v, v-h,* and *h-x* intervals, and, therefore, a percentage decrease in the case of each; (3) an increase in length of the *v-a, h-a,* and *x-a* interval with also a percentage increase less than the actual; (4) an increase in the length of first and second sounds, mainly accounted for by an earlier occurrence before the beginning and the crest of the *c*-wave; (5) an increase in the length of the first to second heart sound interval with a percentage decrease in the length of the cycle; (6) an increase in both actual and percentage lengths of the third to first heart sound interval; and finally (7) both an actual and percentage decrease of the length of the second to third heart sound interval. It will be noted that the second to third heart sound interval differs markedly from the other two heart sound intervals in that with an increase in the length of the cycle, it shows not only a percentage decrease, but an actual decrease. In this actual decrease it differs also, as will be seen, from the features of group II, classed as constant features.

It would seem, indeed, to be a variable feature, dependent to a considerable extent on the length of the cycle but varying inversely with the other features of the cardiac cycle. In other words, the longer the cycle, the shorter the second to third heart sound interval. We have no explanation of this fact; indeed our knowledge of the cause of the third sound is so meager as to make valueless any speculation with regard to it. The variations in length of systole and diastole have already been considered.

The effect of increase in pulse rate as a result of exercise is shown in the fourth series of table I. With an increase of the pulse rate, there is a disappearance of the *h*-wave, and a marked increase in the *a-c* interval. The *c-v* interval is shorter in the more rapid pulse, but in percentage the cycle remains fairly constant. The *c*-wave is actually shorter while its percentage length is greater. Both the first and second heart sounds are of shorter duration in the more rapid pulse, the second sound being especially short, while the first sound begins a shorter interval before the *c*-wave. The third heart sound, on the other hand, is increased in duration and occurs earlier in the cardiac cycle, the second to third heart sound interval undergoing both actual and percentage shortening. It will be noted that this is exactly the reverse condition of that which occurs in cardiac cycles of different length occurring independently of exercise, while in the other features mentioned there is apparently complete correspondence.

The first to second heart sound interval is shorter in the more rapid pulse, while its percentage length is greater. The third to first heart sound interval undergoes both actual and percentage shortening. Systole is shorter but forms a greater percentage of the cardiac cycle, while diastole is shorter both in actual length and in percentage.

The Length of Systole and Diastole.—The determination of the length of systole and diastole and their relation to the total length of the cardiac cycle has been the subject of numerous investigations. The methods employed have been mainly three: (1) the interval from the beginning of the first to the beginning of the second heart sound (Derders, Edgren, Einthoven and Geluk, and others); (2) the interval between the primary and dicrotic elevations on the

arterial pulse curve (Thurston and others); and (3) the interval between the foot of the ascending limb of the cardiogram and the beginning of the descending limb (Hürthle). In regard to the first method, Hürthle (9), and Einthoven and Geluk (10), have shown that at the apex the first sound begins a considerable and variable period before the beginning of the cardiogram, and hence any use of such data as a source of determination of systole is open to this error. Both the dicrotic elevation in the pulse curve and the end of the plateau of the cardiogram represent events which do not seem to be as fixed in position nor as exact in relation to the closure of the semilunar valves at the end of systole, as the sound produced by the closure of these valves; namely, the second heart sound. For these reasons, systole was measured in the present work as the interval between the beginning of the *c*-wave of the venous pulse and the beginning of the second heart sound, plus the period of transmission. The latter was determined by measurement of the separation of the foot of the ascending limb of the cardiogram and the *c*-wave on simultaneous tracings of the venous pulse and apex beat. It has been shown by Hürthle (9), by simultaneous records of intraventricular pressure and apex beat, that if care is used in interpreting cardiographic tracings, the actual point of beginning of rise of intraventricular pressure (onset of systole) may be accurately placed. Finally, Fredericq (2) has shown that the beginning of the *c*-wave, when recorded from the auricle or from the vein (when correction for transmission of the venous pulse is made), is exactly synchronous in its beginning with the foot of the ascending limb of the curve of intraventricular pressure.

The exact meaning of systole has been considered differently by different investigators, and it is well to define at once what is understood in this case. According to Hürthle (11), it is the interval between the beginning of the contraction of the ventricle and the cessation of outflow from the ventricle. The beginning of systole is indicated by the first rise of intraventricular pressure, the end by the closure of the semilunar valves. More accurately stated, the end of systole corresponds with the instant at which intraventricular and aortic pressures become equal. The latter definition is the one adopted in the present work. According to this interpretation,

systole includes the period of tension ("Anspannungszeit"), the period of outflow, and a part, but not all of the period of detension ("Entspannungszeit").

The data given in the table indicate, as has been explained, a point which has been emphasized by most writers on the subject, the relative constancy in length of systole with variations in length of the cardiac cycle. While somewhat longer in longer cycles, the increase in length is not sufficient to maintain a constant percentage. Systole, from the data here given, forms an average length of about 36 per cent. of the total cycle. In individuals with a rapid heart, it may rise to over 38 per cent., or in slow hearts it may form only about 31 per cent. of the total cycle. The shortest percentage noted in any of the records was 28 per cent., the longest 42.5 per cent.

VARIOUS PULSE AND HEART SOUND RECORDS FROM DOGS.

In two experiments, records were obtained from dogs. The venous heart sounds were placed in the auricle, so correction for transmission was not necessary. Figure 3 shows two cycles of the venous pulse and heart sounds in a dog. The third sound does not show in these cycles, but has been recorded a number of times with a slower heart rate. All the records from dogs show a considerable interval (averaging about 0.07 of a second) between the beginning of the first heart sound and the *c*-wave, and since Fredericq (2), (12) has shown that the beginning of the *c*-wave in the auricle is exactly synchronous with the beginning of rise of intraventricular pressure, it is evident that the first sound in the dog begins a considerable time before the beginning of ventricular systole.

The work of Fredericq and others has shown the essential agreement of the venous pulse in man and dog. Measurements of the records obtained in the present work show a fairly close correspondence to the measurements of the records obtained from man, with the main difference that the cardiac cycles obtained from an anesthetized dog with a sound in the auricle are usually much shorter.

A number of records were made of the carotid pulsation and

heart sounds in dogs without anesthesia or operation, the rapid heart rate that usually results from the introduction of a sound into the auricle being avoided in this way. Under these circumstances, the third sound is well recorded, and the heart sound intervals approach closely the length observed in man. An example is given in figure 4.

THE RELATION OF THE CARDIOGRAM AND CAROTID TRACINGS TO THE HEART SOUNDS AND VENOUS PULSE.

In table II are given averages from measurements of the relation between the cardiogram and venous pulse and cardiogram and carotid tracings from the same individuals who served as subjects for the other records. With the data from this table in connection with the data on the relation between the heart sounds and venous pulse in table I, we may construct a diagram representing the average relations of these features of the cardiac cycle. The considerable size of the figures in the columns under total variations in table I indicates that in any individual case, however, there may be considerable variation in the relative position of the various venous pulse waves from the average relations. The *x*-wave is not indicated in the diagram, because it is a wave which is present only in cardiac cycles considerably longer than the average.

SUMMARY.

This investigation comprises a study of venous pulse, carotid, cardiographic, and heart sound records from twenty-one men with normal hearts. The heart sounds were recorded by the method of Einthoven. When correction is made for transmission, the first heart sound begins, on an average, 0.131 of a second before the *c*-wave of the venous pulse, and has an average duration of 0.128 of a second. The second heart sound begins a few hundredths of a second before the *v*-wave and has a duration of 0.091 of a second. The third heart sound occurs shortly before or is coincident with the beginning of the *h*-wave. It has an average duration of about 0.03 of a second. Considerable variation from the average positions are noted in individual cases. This is especially true in the relation between the second heart sound and the *v*-wave.

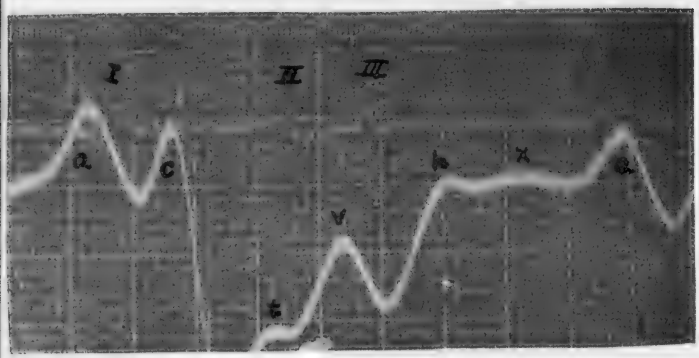

FIG. 1.

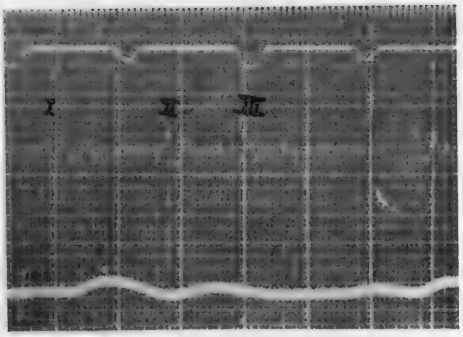

FIG. 4.

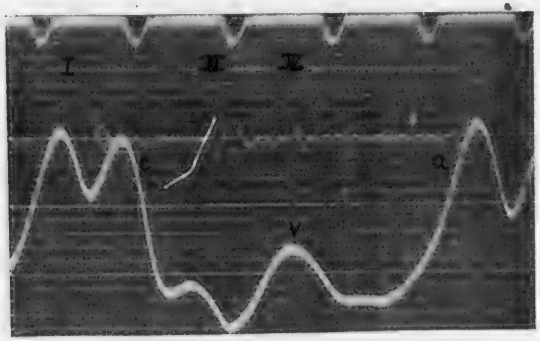

FIG. 2.

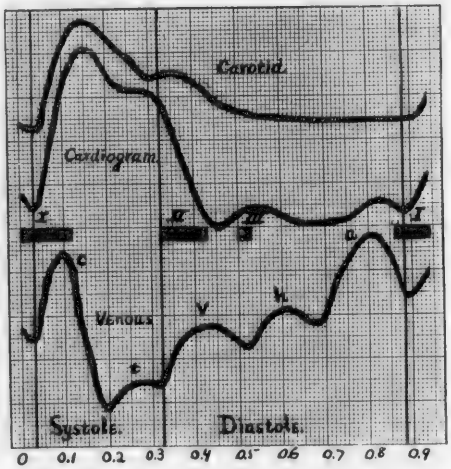

FIG. 5.

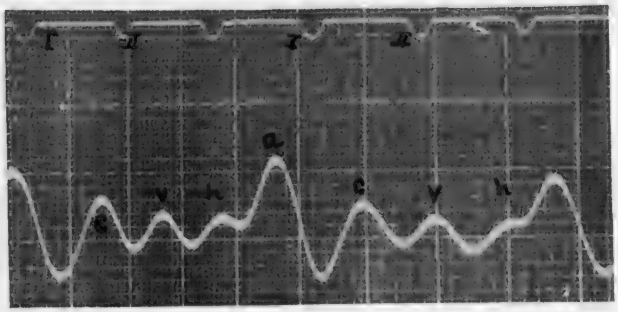

FIG. 3.

Measurements of the venous pulse and heart sound intervals show that the *a-c*, *c-v*, and *v-h* venous pulse intervals, the first to second and the second to third heart sound intervals, and systole tend to remain fairly constant in length when cardiac cycles of different length are considered. The *v-a*, *h-a*, and *x-a* venous pulse intervals, the third to first heart sound interval, and diastole tend to vary in length with variations in total length of the cycle.

Records of the venous pulse and heart sound obtained from dogs show a similar relation to that found in man. The main difference observed was an earlier occurrence of the first heart sound before the *c*-wave of the venous pulse.

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EXPLANATION OF PLATE 53.

FIG. 1. Venous pulse and heart sounds in man. The marks at the top of the record indicate intervals of one hundredth of a second.

FIG. 2. Venous pulse and heart sounds in man. Time in intervals of one fifth of a second.

FIG. 3. Venous pulse and heart sounds in the dog. Time in intervals of one fifth of a second.

FIG. 4. Carotid pulse and heart sounds in the dog.

FIG. 5. The average relations of venous pulse, carotid pulse, cardiogram, and heart sounds in man.

The venous and carotid tracings are corrected for transmission.

THE BIOCHEMISTRY OF BACILLUS LEPRÆ.*

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Since the cultivation, about two years ago, of the leprosy bacillus, increased attention has been directed to the study of immunity processes in infection caused by this microörganism. Experiments which aim at the development of some method for the specific treatment of the disease have already been undertaken by Dr. Duval and his associates in this laboratory, and by Clegg in the Philippines. The present study of the chemical components of *Bacillus lepræ* has been made in order that a more analytic examination of the immunity-inducing substances present in the microörganism may be carried out. We believe that even though the results obtained in therapy with injections of killed bacillary suspensions prove efficacious, it will be necessary to test the relative value of extracts prepared in different ways. The present work will indicate the constituents of the organism found in such extracts as are now employed in the treatment of human leprosy.

Our investigations have been confined chiefly to the quantitative determination of water, fats, lipoids, and proteins. The components of certain of these groups have been identified and estimated, and their relative toxicity has been tested. Our studies serve further to differentiate from *Bacillus tuberculosis* the acid-fast bacillus isolated by Dr. Duval from human leprous lesions.

Contrary to a preconceived notion, we have found that notwithstanding the moist appearance of fresh leprosy cultures as compared with those of the tubercle bacillus, the constant dry weight of the former is relatively higher than that of the latter. According to Hammerschlag,[1] whose findings have been corroborated by many other observers, the average water content of the tubercle

* Received for publication, July 13, 1911.

[1] *Centralbl. f. klin. Med.*, 1891, xii, 9.

bacillus is 85.9 per cent. The constant weight of *Bacillus lepræ* varies considerably according to the moisture present in the medium upon which it is grown, and is found to range from 21.2 to 34.6 per cent. of the total moist weight.

Method.—The cultures tested were grown upon alkaline fish agar[2] containing 3 per cent. glycerin agar, and were scraped from the surface by means of a heavy platinum loop. They have been under cultivation for more than fourteen months, so that a moderately profuse growth is obtained in five or six days. Ordinary culture tubes in which the medium is solidified in the sloped position and poured into Petri dishes have been used in cultivating the organisms.

Upon removal of the growth material from the surface of the agar, it was placed in glass dishes in very thin layers, weighed, and subsequently dried in an air bath for forty-eight hours at a temperature of 62° C. Heating at this temperature for forty-eight hours has been found to bring about constant weight of the growth and does not interfere with the morphology and staining properties. Moreover, although this treatment suffices to kill the bacilli, their toxicity and sensitizing properties are not markedly lessened, as has been shown by animal experiments. When dried in this manner, the material consists of an almost transparent golden brown substance resembling toffee in appearance and being of a similar brittle consistence.

Fat.—In the determination of fat, the dry material was extracted twenty times with cold, and twenty times with boiling acetone. The various extracts were then united, the acetone distilled off, and the residue dried over sulphuric acid to constant weight. The final product had a characteristic acrid odor and consisted of a deep brown, viscous, semi-fluid mass, which, on microscopic examination, was found to contain a few slender, prismatic crystals. 3.95 grams of the dry bacilli yielded 1.37 grams, or 34.7 per cent. of extract.

The presence of free fatty acids could be demonstrated, while an immediate blackening with osmic acid indicated the presence of the glycerids of oleic acid. On saponifying 1.2 grams of the bac-

[2] *Jour. Exper. Med.*, 1911, xiii, 365.

terial fat with alcoholic potash and subsequently extracting the alkaline solution with ether, 0.001 of a gram of a fatty material was obtained, which, on the application of the usual qualitative color tests, gave positive reactions for cholesterol.

The residue left after extraction with acetone was treated fourteen times with boiling chloroform, which, upon distilling off the solvent, permitted the recovery of a small amount of material. This was dissolved in choloroform and reprecipitated by the addition of an excess of acetone. The precipitate, which consisted of a pale yellow, waxy solid, weighed, after drying over sulphuric acid, 0.07 of a gram (equivalent to 1.1 per cent.), and was found to contain phosphorus and to possess the properties of lecithin.

Protein.—After treatment with chloroform, there remained 2.5 grams of a pale yellow material which was almost insoluble in water and in cold dilute acids, but readily soluble in a solution of dilute potassium hydroxid from which it was not precipitated by the addition of acetic acid. This material reacted positively with the biuret, molish, and xanthoproteic tests, with the Hopkins and Cole tryptophan test, and with Millon's reagent, thus proving the presence of a carbohydrate radical, and of tryptophan, tyrosin, and other members of the aromatic series. Sulphur and phosphorus are both demonstrable, the latter in considerable amounts. Nitrogen is present to the extent of 8.20 per cent.

A small quantity of the fat-free portion of the bacilli was boiled with hydrochloric acid until dissolved. The solution was then saturated with ammonia, and formed, on the addition of an ammoniacal solution of silver nitrate, a flocculent precipitate of the silver compounds of the nuclein bases.

Carbohydrates.—Watery extracts of *Bacillus leprœ* give no reduction with Fehling's solutions. Levene[3] has found in the tubercle bacillus a glycogen-like body soluble in water and in dilute salt solutions; but an attempt to isolate a similar substance from *Bacillus leprœ* was unsuccessful.

Coloring Matter.—The greater part of the material which gives to cultures of *Bacillus leprœ* their bright yellow tint belongs to the group of lipochromes. The substance is soluble in all the ordi-

[3] *Med. Rec.*, 1898, liv, 873.

nary fat solvents, such as ether, alcohol, acetone, chloroform, etc., and gives with concentrated sulphuric acid and with nitric acid the color changes characteristic of this class of dyes. There is also present another yellow coloring matter which is insoluble in the solvents used for fat extraction, and which may be extracted with water from the residue left after treatment with chloroform.

A summary of the composition of moisture-free bacilli is given in the following table.

Acetone-soluble extract	34.7 per cent. (neutral fats, free fatty acids, cholesterol.)
Acetone-insoluble, chloroform-soluble extract	1.7 per cent. (lecithin.[4])
Residue	63.6 per cent. (nucleo-proteid, nucleic acid, ash.[5])

Two strains of the leprosy bacillus have been tested for lecithin by methods similar to those described above. In general, the variations obtained are not more marked than can be readily explained by differences in media and errors of experiment.

The fatty content of the tubercle bacillus has been differently estimated by several observers. The variations noted have usually been from 22 to 39.6 per cent., the latter figure representing the benzol-soluble material. Hammerschlag, using alcohol and ether as extractives, recovered 26.2 per cent. of the total dry weight of the bacillus. Klebs,[6] using the same solvents, found but 22 per cent. De Schweinitz and Dorset[7] alone have been successful in isolating as much as 39.6 per cent.

By most investigators, cholesterol is said to be absent, and, as far as we have been able to discover, it has been identified by none.

[4] Preliminary estimations of lecithin resulted in a large percentage of this material, which may be accounted for by differences in the media used in growing the organisms.

[5] Although owing to scarcity of material, actual quantitative estimations of the ash have been impossible, it is evident that a very small portion consists of this substance.

[6] *Centralbl. f. Bakteriol., 1te Abt.*, 1896, xx, 488.

[7] *Jour. Am. Chem. Soc.*, 1895, xvii, 605.

Hammerschlag has found minute quantities of a phosphatid fat, but his observations have not been confirmed.

TABLE I.

Protocols of Mice Inoculated with Leprous Protein and Dead Bacilli.

Animal.	Dose.	Material.	Result.
Full grown mouse	1.00 c.c.	4 per cent. L.P.*	Died in 6 hours.
Full grown mouse	0.50 c.c.	4 per cent. L.P.	Died in 6 hours.
Full grown mouse	0.25 c.c.	4 per cent. L.P.	Died in 6 hours.
Full grown mouse	0.25 c.c.	4 per cent. L.P.	Died in 6 hours.
Full grown mouse	0.10 c.c.	4 per cent. L.P.	Very sick for 36 hours; recovery.
Full grown mouse	0.05 c.c.	4 per cent. L.P.	No apparent symptoms.
Full grown mouse	0.05 c.c.	4 per cent. L.P.	No apparent symptoms.
Full grown mouse	0.05 c.c.	4 per cent. L.P.	No apparent symptoms.
Full grown mouse	0.50 c.c.	4 per cent. D.L.*	Sick 24 hours; recovery.
Quarter grown white mouse	0.50 c.c.	4 per cent. D.L.	Died in 30 hours.
Full grown white mouse	0.50 c.c.	4 per cent. D.L.	No apparent symptoms.
Full grown white mouse	0.25 c.c.	4 per cent. D.L.	No apparent symptoms.
Full grown white mouse	0.10 c.c.	4 per cent. D.L.	No apparent symptoms.
Ful grown white mouse	0.05 c.c.	4 per cent. D.L.	No apparent symptoms.

* L.P. = leprous protein; D.L. = dead bacilli.

The essential chemical differences between the leprosy bacillus and the tubercle bacillus are the relatively higher water content of the latter and the presence of comparatively large quantities of lecithin and cholesterol in the former.

Toxicity.—Experiments have been performed with a view to determining the relative toxicity of the bacillary constituents as compared with the whole organism. It should be stated that like the tubercle bacillus the essential toxicity of the leprosy bacillus is very low. The observation of human cases harboring enormous numbers of the bacilli without showing symptoms of specific intoxication proves this fact. That the bacillus does, however, possess a certain degree of toxicity, and that the protein content is the source of poison, is shown by the following experiments.

Into the peritoneal cavity of fifteen white mice were inoculated simultaneously a suspension of dead bacilli, killed by heating to 62° C. for forty-eight hours, and a suspension of leprous protein. Both suspensions contained 4 per cent. by weight of the dried material.

The effect produced by the inoculation of both dead bacilli and leprous protein appears to be of the nature of collapse. The animals huddle together in the corner of the cage and can not be induced to move. Respiration becomes accelerated and the animal falls into a stuporous condition and dies.

At autopsy, with the exception of a slight peritoneal change, no lesions of any kind were seen in any of the animals injected with leprous protein. However, in the peritoneal cavity of each animal there was found, about the site of inoculation, a localized area of slightly brownish discoloration, which was apparently the remains of injected material. On microscopical examination, the small amount of material present in these areas was seen to contain no fibrin.

The mice dying as the result of inoculation with dead bacilli showed a more marked lesion of the peritoneum, as was evidenced by the presence of a moderate amount of exudate. Microscopically, the exudate contained fibrin and cells chiefly of the polymorphonuclear type, together with a few large mononucleated cells. The former as well as many of the latter were filled with leprosy bacilli.

From these experiments we conclude that the protein portion of the organism represents practically the whole of the toxic body. Indeed, it appears that instead of destroying the potency of the toxic property of the bacillus, the freeing of the organism from its fatty covering renders the toxin more absorbable so that it acts more quickly and effectively.

THE RELATION OF HEMATIN TO PATHOLOGICAL PIGMENT FORMATION.*

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Much of the literature dealing with the subject of the hematogenous pigments, hemosiderin and hematoidin, is peculiarly silent upon the question of the relation of these pigments to hematin. In considering the origin of hemosiderin and hematoidin, most authors have contented themselves with establishing the fact that these pigments are derived from hemoglobin, and, although the formation of hemosiderin is usually ascribed to a process of vital oxidation, there is little agreement as to the exact mode of their production, or as to their genetic relation to other pigments.[1]

Nevertheless, definite statements of the relation of hematin to these pigments are not entirely lacking, particularly in text-books. To present the matter more exactly, a recent text-book in dealing with this subject contains the following statement:[2] "As a pathological pigment, however, hematin is by no means so frequently found as its derivatives. Wherever formed, its duration is transient, for it gradually splits up into an iron-free pigment (hematoidin) and an iron-containing pigment (hemosiderin). This change may be represented by the following equation, according to Nencki and Sieber:

$$\underset{\text{(Hematin)}}{C_{32}H_{32}N_4O_4Fe} + 2H_2O = 2\underset{\text{(Hematoidin)}}{C_{16}H_{18}N_2O_3} + Fe."$$

The evidence in support of this conception might be summarized as follows: (1) that increased destruction of red blood corpuscles

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[1] For a full discussion of this question, see Neumann, *Virchows Arch. f. path. Anat.*, 1904, clxxvii, 401.

[2] Wells, Chemical Pathology, Philadelphia and London, 1907, 401.

or of hemoglobin, within the circulation, leads inevitably to an increased production of bile pigments and a deposit of hemosiderin in the spleen, bone marrow, liver, and other organs; (2) that the local metabolism of red blood corpuscles or of hemoglobin results in the formation of hemosiderin or hematoidin, or both; (3) that proteolytic enzymes split hemoglobin into proteid and hematin; and finally, (4) that the hydrolytic cleavage of hematin, by splitting off the iron, results in the formation of a pigment (hematoporphyrin) essentially the same as hematoidin.

Although there are obvious defects in such a chain of reasoning, it must be admitted that, knowing the fate of hemoglobin and that it is capable of cleavage into proteid and hematin and that the hydrolytic cleavage of hematin results in the formation of hematoidin plus iron, it is difficult to escape the conclusion that both hemosiderin and hematoidin are at least potential derivatives of hematin. The conclusive proof of the truth or fallacy of this statement would seem possible of experimental demonstration, and yet it appears that the crucial test has never been applied. In the present investigation, an attempt has been made to determine the relation of hematin to hemosiderin and hematoidin upon a more accurate experimental basis. With this object in view, experiments were carried out along three different lines: (1) the intravenous injection of alkaline hematin solutions in the rabbit, and intraperitoneal injection in the guinea pig; (2) the subcutaneous and intraperitoneal injection of powdered hematin and crystalline hemin in the guinea pig; and (3) the intraperitoneal injection of powdered parahemoglobin in the guinea pig.

The hematin and hemin used were prepared, according to the Schalfijew process,[3] from rabbit and from guinea pig blood. The parahemoglobin was prepared from rabbit's blood by thoroughly washing the red blood corpuscles with 0.85 per cent. salt solution, shaking the corpuscles in a separatory funnel with ether and distilled water, and then filtering the hemoglobin solution. This was evaporated to dryness at a temperature between 37° and 40° C. The dry residue was then ground to a coarse powder in a mortar and placed under absolute alcohol for one week, at room tempera-

[3] Schäfer's Text-Book of Physiology, Edinburgh and London, 1898, i, 252.

ture. At the end of this time the alcohol was decanted, the residue washed with ether and, after drying thoroughly, ground to a fine powder. This powder was absolutely insoluble in either distilled water or 0.85 per cent. salt solution, and very sparingly soluble in dilute alkalies.

The injections of the powdered hematin, crystalline hemin, and powdered parahemoglobin were all accomplished by suspending these substances in sterile 0.85 per cent. salt solution, the injections being made under aseptic precautions.

While the first series of experiments, that of intravenous injection of dissolved hematin, gives valuable information as to the toxicity and fate of the pigment thus injected, complications are introduced by the action of the solvent and the extreme toxicity of such hematin solutions, which render the series of experiments unsuitable for the object of this inquiry. I shall, accordingly, devote this paper to recording the results obtained by the other two methods.

The above complicating circumstances are reduced to a minimum by the use of powdered hematin suspended in salt solution. Therefore, as extravasated blood and hemoglobin injected into the tissues have been shown to be subject to the same metabolic changes as red blood corpuscles destroyed within the circulation, or as hemoglobin injected into the circulation, the use of hematin suspended in salt solution would seem to be the method apt to give the most reliable results. It might be objected, however, that the use of powdered hematin does not simulate normal or pathological conditions upon which we have reliable data as to the fate of hemoglobin. To meet such objections, the powdered parahemoglobin was introduced as a control. It remains, therefore, only to determine whether this parahemoglobin can be converted into hemosiderin or hematoidin by the tissue cells, and finally, whether or not powdered hematin is subject to the same metabolic changes.

When powdered parahemoglobin suspended in salt solution is injected, under aseptic conditions, into the peritoneal cavity of the guinea pig, it collects principally in the omentum and about the point of puncture in the abdominal wall, although, to a less degree, it is distributed throughout the abdominal cavity. The ensuing reaction about the particles of pigment is essentially that which occurs

about a sterile, non-absorbable, foreign body, the giant cell being a prominent feature of the early stages of the reaction. No appreciable absorption of the pigment seems to take place, and although much of the finer pigment is picked up by phagocytic cells, these appear so sharply focalized as to render it doubtful if much removal of pigment from the peritoneal cavity is accomplished through this agency. At the end of five days, many granules of parahemoglobin have been partially or wholly converted into copper-colored pigment which exhibits a typical iron reaction, while other granules remain unchanged in character, as far as can be determined. As time progresses, more and more of the parahemoglobin is converted into definite hemosiderin granules. Under these conditions, however, in no instance has any demonstrable hematoidin been noted as a product of the action of the tissue cells upon powdered parahemoglobin.

It is certain, therefore, that the physical condition of parahemoglobin cannot prevent its transformation into hemosiderin. There is undoubtedly a retardation of the process and possibly also a less perfect conversion into hemosiderin, both of which are readily ascribable to the physical condition of parahemoglobin as compared with the natural state of this pigment.

An attempt was next made to determine whether a like change was produced in powdered hematin in the peritoneal cavity and subcutaneous tissues. In a series of experiments, the duration of which ranged from five to forty-seven days, the results were so uniform that they can be rendered sufficiently clear by extracts from a few protocols of typical cases from various stages of the experiments.

Experiment 1.—White female guinea pig, weight 500 grams. On January 7, 1911, 15 mg. of guinea pig hematin, suspended in salt solution, were injected into the peritoneal cavity. The animal remained normal, and after five days was killed by a blow on the head.

Autopsy.—The subcutaneous tissues about the site of the injection showed slight edema and ecchymosis, and a small amount of black pigment. The abdominal cavity contained a large amount of black pigment distributed irregularly over all the peritoneal surfaces, but collected especially about the site of puncture in the abdominal wall, and in the rolled up omentum. The aggregates of pigment varied from a diffuse smear to perfectly discrete nodules, and the diameters of the latter varied from that of a pin point to 0.5 cm. The larger foci, espe-

cially on the abdominal wall, showed an areola of redness about the black pigment. The pigmented nodules were adherent to the surfaces upon which they rested, though they could be rubbed off with force. With the exception of a slight enlargement of the spleen and lymph nodes, other conditions were normal.

Microscopic examination showed that the larger nodules of pigment were composed of fine and coarse, irregular masses of black pigment thickly imbedded in newly formed granulation tissue. There was pronounced hemorrhage throughout the nodules and in the tissues about their periphery. The granulation tissue contained many giant cells encircling the coarser masses of pigment, while there were numerous polymorphonuclear leucocytes at the centers of the nodules and many mononuclear phagocytes containing fine granules of brown and black pigment. Here and there through the nodules, but more especially in the adjacent tissues, were phagocytic cells containing yellowish brown pigment. Very few phagocytes containing black pigment could be detected in the surrounding tissues.

The smaller nodules differed from the larger ones only in showing little or no hemorrhage, very few leucocytes, and a very slight amount of yellowish brown pigment.

On applying the Prussian blue test for iron, a diffuse blue color was developed throughout the larger nodules of pigment, usually more pronounced at the periphery than at the center of the nodule. There were many fine granules of pigment which gave a positive iron reaction within the nodule, while most of the yellowish brown pigment in the surrounding tissues reacted positively. The smaller nodules of pigment showed only a few scattered granules which manifested an iron reaction, and the diffuse reaction was very slight indeed. The black pigment in all nodules still showed the characteristic solubility of hematin and did not react for iron by the usual technique.

Experiment 2.—Male guinea pig, weight 289 grams. On January 27, 1911, 15 mg. of rabbit hematin were injected into the peritoneal cavity. The animal remained normal and was killed after fifteen days.

Autopsy.—A large amount of black pigment was found distributed through the peritoneal cavity as in experiment 1, but there were fewer large nodules of pigment. The omentum was not rolled up, and there were no reddened zones about the nodules of black pigment.

Microscopically, the pigmented nodules were composed principally of masses of black granular pigment imbedded in granulation tissue and immediately surrounded by giant cells. There were numerous mononuclear phagocytes present which contained either fine granules of yellowish brown pigment or granules of black pigment, the latter being far more numerous than the former. Some of the larger masses of black pigment showed paler spots or borders and there were occasional granules of a greenish brown color. There were also a few almost colorless refractile bodies, the general morphology of which resembled that of the hematin masses. There was no appreciable hemorrhage and but few polymorphonuclear leucocytes.

The Prussian blue test for iron, on a slide, showed in addition to a very slight diffuse bluish coloration, two types of pigmented bodies manifesting a positive reaction for iron. Bodies of the first type consisted of yellowish brown granules and gave a deep Prussian blue color to the entire granule.

These were few in number and were found particularly about the periphery of the nodules. Granules of the second type had a similar color, but were slightly more refractile, and gave a reaction in which the blue precipitate spread away from the granule, or was precipitated about the granule, the body of the granule not becoming colored, but retaining its original yellowish brown color. The almost colorless refractile bodies referred to above, exhibited this type of iron reaction, though some of these (the least pigmented) showed the blue body of the granule. Sections, washed and mounted, showed a persistence of the Prussian blue in the pigments of the first class, though the blue was largely washed away from those of the second class. Such masses of hematin as showed evidence of bleaching, as above described, and those granules which had assumed a greenish brown color, showed varying degrees of an iron reaction of the second type, perfectly colorless areas giving an intense blue reaction.

The black pigment throughout the section still showed the characteristics of hematin.

Experiment 3.—Male guinea pig, weight 370 grams. On January 27, 1911, 20 mg. of rabbit hematin were injected into the peritoneal cavity. The animal remained normal for forty-five days and died from pneumonia on the forty-seventh day.

Autopsy.—The autopsy was done two hours after death. The peritoneal cavity presented an appearance similar to that of experiment 2, except that there appeared to be a greater amount of black pigment which was collected in larger nodules.

Microscopically, the pigmented nodules, although similar in general appearance, showed certain differences from those previously described. The granulation tissue was denser and formed a definite fibrous capsule about many clumps of pigment. The larger nodules again showed hemorrhage and a considerable number of polymorphonuclear leucocytes. For the most part, the pigment masses were broken up into smaller granules although there were still numbers of large masses surrounded by giant cells. There seemed to be no reduction in the total amount of hematin present, although the changes were more evident and there were numerous small granules which were almost completely decolorized, as well as larger pigment masses showing partially or completely decolorized margins. In addition to the hematin, the hemorrhagic nodules showed a small amount of yellowish brown pigment which was very scarce in the nodules without hemorrhage.

The Prussian blue reaction again showed a diffuse blue in the hemorrhagic nodules which was much less pronounced where there was no hemorrhage. The yellowish brown granules of hemosiderin were most numerous where there was hemorrhage, and almost completely absent where there was no hemorrhage. The partially and completely decolorized granules and portions of hematin masses gave an iron reaction, the nature and intensity of which was proportional to the degree of decolorization, as explained in experiment 2.

The changes produced in hematin in the subcutaneous tissues are absolutely identical with those produced in the peritoneal cavity, and, therefore, require no detailed presentation.

To reduce these experimental results to more general statements,

it can be said that, post mortem, the hematin introduced into the peritoneal cavity appears as black pigment distributed over all the peritoneal surfaces, being most abundant in the omentum and about the point of injection, frequently forming nodules as large as 0.5 of a centimeter in diameter. Wherever the hematin lodges, it is rapidly walled in by new growth of connective tissue and foreign body giant cells. The reaction on the part of the tissues is further characterized by a tendency to hemorrhage, both around and within the connective tissue nodules formed. This hemorrhage may persist in the larger nodules or recur at intervals for weeks, but it does not involve all nodules, especially the smaller ones where, very early, the hemorrhage usually disappears completely. The leucocytes present in the early stages of the experiments gradually give place to mononuclear cells of the polyblastic series. At the same time, there is an increase in the density of the connective tissue and the formation of a definite fibrous capsule about some nodules. Grossly, there is no apparent difference in the amount of hematin found at different stages of the experiments.

Microscopically, the hematin stands out as the most prominent feature of the section in all stages of the experiments. This pigment occurs as large irregular masses, mostly surrounded by giant cells, and as a finely granular black pigment, much of which is being picked up by phagocytes. There is but slight evidence, however, of removal of pigment from the peritoneal cavity, as only a very small amount can be found in the mesenteric lymph nodes, spleen, liver, or other organs.

In the early stages, no change is demonstrable in the hematin. Later, the larger masses are slowly broken up and there is increased phagocytosis. At the same time, the small granules of hematin and the margins of the larger masses begin to show a diminution of color intensity, giving rise to a series of pigmented bodies varying from dark brown or greenish brown through lighter shades of brown—yellowish brown and straw yellow—ultimately becoming perfectly colorless and highly refractile. At the end of forty seven days, the total change produced is but slight, by far the greater part of the hematin showing no demonstrable alteration.

Morphologically, these pigmented derivatives of hematin are

practically indistinguishable from certain varieties of hemosiderin, which, according to Schmidt[4] and to Neumann,[5] may show an even greater variety of color changes in its development and subsequent decomposition. The analogy becomes closer when to these morphological characters is added the fact that these modified products of hematin exhibit a microchemical reaction for iron by the Prussian blue method. This reaction, however, shows certain differences from that of hemosiderin. It will be recalled that the typical hemosiderin reaction results in the formation of a solid blue granule in which none of the original color of the pigment is perceptible. In addition to this type of reaction, Schmidt describes a second type, exhibited by what might be termed incompletely developed hemosiderin. This reaction is characterized by a precipitation of Prussian blue about the pigment, the original color of which is still distinctly visible. It is this second type of reaction that is shown by the colored products derived from hematin, the reaction approximating the typical hemosiderin reaction as the color of the granule decreases, and becoming typical only in the completely decolorized products of hematin, in which respect the reaction is decidedly different from that of hemosiderin.

This peculiarity of the iron reaction led to further comparison of the properties of hemosiderin and the pigmented hematin derivatives. On testing the solubility of these pigments in fixed tissue, it was found that whereas hemosiderin is insoluble in dilute alkalies (0.2 per cent. potassium hydrate), and, except for a slight brightening in color, shows no demonstrable change, the darker hematin derivatives are practically completely dissolved and the color is extracted from the lighter bodies, leaving a colorless residue which reacts for iron exactly as the originally colorless derivatives. A further difference between the two classes of pigment is shown by their behavior in the presence of hydrogen peroxid.[6] While the hematin derivatives suffer complete destruction after a few hours' action of this reagent, hemosiderin remains unaffected for several days. These tests, then, furnish further means for the

[4] Schmidt, *Virchows Arch. f. path. Anat.*, 1889, cxv, 397.
[5] Neumann, *loc. cit.*
[6] Brown, *Jour. Exper. Med.*, 1911, xiii, 477.

positive identification of hemosiderin, and, in the present instance, serve to show conclusively that these pigmented derivatives of hematin, though closely analogous, are not true hemosiderin but are bodies of a distinctly different nature which, for convenience, might be termed hemosideroid pigments.

Intimately associated with the hematin and the hemosideroid pigments in the peritoneal cavity and the subcutaneous tissues, there is usually present a variable amount of typical hemosiderin. This pigment is more constant in the early stages of the experiments, while later it appears only in such nodules as show the presence of hemorrhage. In no instance, however, has there been any evidence of the production of hematoidin.

The series of animals in which crystalline hemin was injected subcutaneously and intraperitoneally gave results which agree in all essential respects with those obtained with hematin. The hemin seems more irritating, and the tendency to hemorrhage in and about the pigment nodules is more pronounced. The hemin injections proved of great value in confirming results obtained with hematin, as the crystalline substance is so characteristic that it can be quite easily identified in the tissues, even after the color has been markedly altered. A single difficulty was encountered in the study of these crystals, and that was the preservation of the crystals in fixing the tissues in which they were imbedded. Tissues, such as the omentum, which permit of microscopic examination, while still fresh may show perfect preservation of the crystalline form, but after fixation with either alcohol or formalin, the crystalline hemin may be largely replaced by a fine brownish black precipitate or fine crystalline needles of the same color. There are usually enough crystals preserved, however, to enable one to trace the changes produced by the tissues, and this can be done best from portions of omentum or mesentery stretched over cover slips before fixation. With such preparations or with the usual sections, it is not difficult to recognize single crystals and groups of crystals with typical hemin morphology which are partially or completely decolorized and exhibit an iron reaction identical with that described for hematin. These altered crystals are also easily soluble in dilute caustic alkalies and easily destroyed by hydrogen peroxid,

as in the case of hematin. It seems certain, therefore, that both of these substances suffer the same type of change in animal tissues.[7]

Another point of interest that should be noted is that there is no difference whatsoever in the action of these products (hematin and hemin) from rabbit blood or from guinea pig blood.

In conclusion, it must be said that hematin, when injected into animal tissues, is not a substance of transient duration but, on the contrary, is metabolized very slowly and with great difficulty. It is of further importance to emphasize the fact that the sole demonstrable change occurring in either hematin or hemin within the tissues is that indicated by a slowly progressing decolorization of these pigments. As this change is absolutely identical with that produced by the oxidation of these substances by hydrogen peroxid, it is but natural to conclude that the process here involved is an oxidation and not a cleavage. Again, as the total amount of hemosiderin produced has always been exceedingly slight compared with the hematin injected, and as this hemosiderin has appeared only where there has been hemorrhage, it is quite clear that the hemosiderin has arisen from extravasated red blood corpuscles and not from the hematin injected. In so far as tissue cells are capable of metabolizing hemoglobin with the production of hemosiderin and hematoidin, while these cells seem wholly unable to form these pigments from hematin, the view that hemosiderin and hematoidin are derived from hematin appears to be untenable, and we are confronted by the necessity of seeking other explanations for the mode of formation of these pigments.

This series of observations, therefore, is significant on account of the light which is thrown on certain aspects of hemoglobin metabolism, especially the production of hemosiderin, as the hemosideroid products of hematin are probably to be regarded as closely analogous to true hemosiderin, the main difference being the presence of a protein group in the latter pigment. This difference, in turn, seems to be due to the fact that one series of pigments is

[7] The author has recently noted that many cover-glass preparations of omentum containing hemin and hematin left in dilute alcohol and exposed to air and sunlight develop these changes more rapidly than in the animal body.

produced by the oxidation of the non-protein portion of hemoglobin, while the other, hemosiderin, is produced by a similar process from the entire hemoglobin molecule, both the protein and hematin being essential to the formation of typical hemosiderin.

As regards hematoidin, so little is known of the circumstances connected with the formation of this pigment that any positive statement, based on these experiments, would seem unwarranted. However, the failure of the tissues to produce any demonstrable hematoidin when coupled with the fact that hematin undergoes a destructive oxidation would seem sufficient to cause some doubt as to the production of hematoidin from hematin according to the scheme of Nencki and Sieber.

SUMMARY.

1. It has been shown that powdered hematin is exceedingly resistant to the metabolic action of tissue cells, and wherever injected it will remain for weeks with but slight and slowly progressing alteration.

2. The changes produced in hematin by tissue cells are identical with those changes resulting from the oxidation of hematin by hydrogen peroxid, forming a series of bodies of decreasing color intensity, which manifest an iron reaction in inverse proportion to their color.

3. These hemosideroid pigments are distinguished from true hemosiderin by the type of their iron reaction, by their solubility in dilute alkalies, and by their destructive oxidation by hydrogen peroxid.

4. Crystalline hematin injected into the tissues is subject to the same changes as powdered hematin.

5. Whereas powdered parahemosiderin is converted into hemosiderin by the tissue cells with comparative ease, powdered hematin shows no such conversion within forty-seven days, and as the only demonstrable change produced in hematin by the tissue cells does not result in the formation of either hemosiderin or hematoidin, the assumption that hematin is the progenitor of these pigments seems unwarranted.

INDEX TO VOLUME XIV.

ABDOMINAL aorta, patching of, with a piece of rubber, 126
Acid salvarsan solutions, the formation of precipitates in the blood *in vitro* by, 179
Acute anaphylaxis, salvarsan and, 497
Adrenalin, absence of, in malignant renal hypernephromas, 550
Adrenalin in blood, so-called biological tests for, 377
Albumen, urinary, composition of, 298
Anaphylaxis, acute, and salvarsan, 497
Anaphylaxis, lethal cardiac, in the rabbit, 476
Anaphylaxis, studies in immunity and, 44
Anaphylaxis, the toxic action of certain normal sera and its relation to, 25
Anti-pepsin, so-called, a chemo-biological study of the relations of pepsin to, 535
Aorta, abdominal, patching of, with a piece of rubber, 126
Apex of the heart, a study of tracings from the region near the, 351
APOLANT, H. The question of athrepsia, 316
Arterial hypertonus, 377
Artery, pulmonary, of the dog, nerve fibrillæ in the, 322
Artery, radial, a study of simultaneous tracings from the apex of the heart and the, with the micrograph, 339
Athrepsia, 316
AUER, J. The effect of salvarsan upon the heart in dogs, 248
——. Lethal cardiac anaphylaxis in the rabbit, 476
——. A note on salvarsan and acute anaphylaxis, 497
AUER, JOHN, and MELTZER, S. J. The status of respiration in the methods of differential pressure compared with that under the method of intratracheal insufflation, 569
AUSTIN, J. HAROLD, and EISENBREY, A. B. Experimental acute nephritis: the elimination of nitrogen and chlorides as compared with that of phenolsulphonephthalein, 366

BACILLUS lepræ, biochemistry of, 606
Bacillus of leprosy, experimental immunity with reference to the, 181
Bacteria, fecal, a method for the quantitative determination of, 433
BIGELOW, J. E. See KING, BIGELOW, and PEARCE, 159
Bile, the pathology of intraperitoneal injections of, in the rabbit, 445
Biliary cirrhosis in the rabbit, 401
Biochemistry of *Bacillus lepræ*, 606
Biological tests, so-called, for adrenalin in blood, with observations on arterial hypertonus, 377
Blood *in vitro*, the formation of precipitates in, by acid salvarsan solutions, 179
Blood platelets, the rate of regeneration of, 265
Blood, so-called biological tests for adrenalin in, with observations on arterial hypertonus, 377
BROOKS, CLYDE. A note on the absence of adrenalin in malignant renal hypernephromas, 550
BROWN, WADE H. The relation of hematin to pathological pigment formation, 612
——. See BUNTING and BROWN, 445
BUNTING, C. H., and BROWN, W. H. The pathology of intraperitoneal bile injections in the rabbit, 445
BURROWS, MONTROSE T. See CARREL and BURROWS, 244

CALCIUM and iron incrustation and other lesions of the elastic tissue of the spleen and liver, 59
Canal Zone, Isthmian, strongyloides infections in man and animals in the, 1
Cancers of the white rat, immunity in, 139
Cardiac anaphylaxis, lethal, in the rabbit, 476
CARREL, ALEXIS. The ultimate result of a double nephrectomy and the replantation of one kidney, 124
——. Patching of the abdominal aorta with a piece of rubber, 126

Carrel, Alexis, and Burrows, Montrose, T. An addition to the technique of the cultivation of tissues *in vitro*, 244
Cecil, Russell L. On hypertrophy and regeneration of the Islands of Langerhans, 500
Chemo-biological study of the relation of pepsin to so-called anti-pepsin, 535
Chemo-immunological studies on localized infections. Third paper: Some further observations upon the action of certain soaps on the pneumococcus and its experimental infections, 256
Chlorides and nitrogen, the elimination of, as compared with that of phenolsulphonephthalein in experimental acute nephritis, 366
Chlorin retention in pneumonia, the mechanism of, 289
Circulation, pulmonary, 326
Circulation, the relation of the optic thalamus to, 408
Cirrhosis, biliary, in the rabbit, 401
Complement deviation in scarlet fever with comparative studies of the Wassermann and Noguchi systems, 235
Composition of urinary albumen, 298
Crehore, Albert C. A study of simultaneous tracings from the apex of the heart and the radial artery with the micrograph, 339
——. A study of tracings from the region near the apex of the heart, 351
——. On the distribution and direction of motion of the interference bands of light formed by thin plates as the thickness of the plate varies, 357
——. A study of the presphygmic period with the micrograph, 420
Crisis, phagocytic immunity in pneumococcus infections and in pneumonia with relation to the, 109
Cultivation of pure pathogenic *Treponema pallidum*, 99
Cultivation of tissue in plasma from alien species, 129
Cultivation of tissues *in vitro* as a method for the study of cytotoxins, 453
Cultivation of tissues *in vitro*, the technique of, 244
Cutaneous reaction in syphilis, 557
Cytotoxic sera, a physiological study of experimental nephritis due to bacterial poisons and, 306
Cytotoxins, the cultivation of tissues *in vitro* as a method for the study of, 453

Darling, S. T. Strongyloides infections in man and animals in the Isthmian Canal Zone, 1
Denis, W. See Gurd and Denis, 606
Determination, quantitative, of fecal bacteria, a method for the, 433
Deviation, complement, in scarlet fever, with comparative studies of the Wassermann and Noguchi Systems, 235
Dextrose, the excretion of, in the stomach and small intestine, 274
Differential pressure, the status of respiration in the methods of, compared with that under the method of intratracheal insufflation. 569
Direction and distribution of motion of the interference bands of light formed by thin plates as the thickness of the plate varies, 357
Distribution and direction of motion of the interference bands of light formed by thin plates as the thickness of the plate varies, 357
Draper, George. See Robinson and Draper, 217
Duke, W. W. The rate of regeneration of blood platelets, 265
Duval, Charles W., and Gurd, Fraser B. Experimental immunity with reference to the bacillus of leprosy. Part I. A study of the factors determining infection in animals, 181

Eisenbrey, A. B. A study of the elimination of phenolsulphonephthalein in various experimental lesions of the kidney, 462
——. See Austin and Eisenbrey, 366
——. See Pearce and Eisenbrey, 306
——. See Pearce, Karsner, and Eisenbrey, 44
Elastic tissue of the spleen and liver, calcium and iron incrustation and other lesions of the, 59
Electrocardiograph, studies with, on the action of the vagus nerve on the human heart, 217
Elimination of phenolsulphonephtha-

lein in various experimental lesions of the kidney, 462
Excretion of dextrose in the stomach and small intestine, 274
Experimental acute nephritis: the elimination of nitrogen and chlorides, as compared with that of phenolsulphonephthalein, 366
Experimental immunity with reference to the bacillus of leprosy, 181
Experimental lesions of the kidney, the elimination of phenolsulphonephthalein in, 462
Experimental nephritis due to bacterial poisons and cytotoxic sera, 306
Experimental obstructive jaundice, 159
Experimental syphilis and yaws in the rabbit, 196
EYSTER, J. A. E. Studies on the venous pulse. II. The time relations of the venous pulse and the heart sounds, 594

FECAL bacteria, a method for the quantitative determination of, 433
Fibrillæ, nerve, in the pulmonary artery of the dog, 322

GURD, FRASER B., and DENIS, W. The biochemistry of *Bacillus lepræ*, 606
GURD, FRASER B. See DUVAL and GURD, 181

HAMBURGER, WALTER W. A chemo-biological study of the relations of pepsin to so-called antipepsin, 535
HANES, FREDERIC M. See LAMBERT and HANES, 129, 453
HAWK, P. B. See MATTILL and HAWK, 433
Heart, a study of simultaneous tracings with the micrograph from the radial artery and the apex of the, 339
Heart, a study of tracings from the region near the apex of the, 351
Heart, human, the action of the vagus nerve on, 217
Heart in dogs, the effect of salvarsan upon the, 248
Heart sounds, the time relations of the venous pulse and, 594
Hematin, the relation of, to pathological pigment formation, 612
Hypernephromas, malignant renal, absence of adrenalin in, 550
Hypertonus, arterial, 377
Hypertrophy and regeneration of the islands of Langerhans, 500

IMMUNITY and anaphylaxis, 44
Immunity, experimental, with reference to the bacillus of leprosy, 181
Immunity in cancers of the white rat, 139
Immunity, intra-stomachal and intra-intestinal inoculations of trypanosome virus with tests for, 526
Immunity, phagocytic, in pneumococcus infections and in pneumonia with relation to the crisis, 109
Incrustation, calcium and iron, and other lesions of the elastic tissue of the spleen and liver, 59
Infection of rabbits with the virus of poliomyelitis, 116
Infections, experimental, the action of certain soaps on the pneumococcus and its, 256
Infections, localized, chemo-immunological studies on, 256
Infections, pneumococcus, phagocytic immunity in, 109
Infections, strongyloides, in man and animals in the Isthmian Canal Zone, 1
Influenzal meningitis, serum treatment of, 73
Injection of salvarsan, intravenous, the formation of precipitates after, 83
Inoculations, intra-stomachal and intra-intestinal, of trypanosome virus with tests for immunity, 526
Insufflation, intratracheal, the status of respiration in the methods of differential pressure compared with that under the method of, 569
Interference bands of light formed by thin plates, the distribution and direction of motion of, as the thickness of the plate varies, 357
Intestine, small, the excretion of dextrose in the stomach and, 274
Intra-intestinal and intra-stomachal inoculations of trypanosome virus with tests for immunity, 526
Intraperitoneal bile injections in the rabbit, the pathology of, 445
Intra-stomachal and intra-intestinal inoculations of trypanosome virus with tests for immunity, 526
Intratracheal insufflation, the status of respiration in the methods of dif-

ferential pressure compared with that under the method of, 569
Intravenous injection of salvarsan, the formation of precipitates after, 83
In vitro cultivation of tissues, as a method for the study of cytotoxins, 453
In vitro, formation of precipitates in blood, by acid salvarsan solutions, 179
In vitro, technique of the cultivation of tissues, 244
Iron and calcium incrustation and other lesions of the elastic tissue of the spleen and liver, 59
Islands of Langerhans, hypertrophy and regeneration of the, 500

JAUNDICE, experimental obstructive, 159
JOSEPH, DON R. On the formation of precipitates after the intravenous injection of salvarsan, 83
——. The formation of precipitates in the blood *in vitro* by acid salvarsan solutions, 179

KARSNER, HOWARD T. Nerve fibrillæ in the pulmonary artery of the dog, 322
——. See PEARCE, KARSNER, and EISENBREY, 44
Kidney and liver, proteins of, 44
Kidney, the elimination of phenolsulphonephthalein in various experimental lesions of the, 462
Kidney, the ultimate result of a double nephrectomy and the replantation of one, 124
KING, JOHN H., BIGELOW, J. E., and PEARCE, L. Experimental obstructive jaundice, 159
KLEINER, ISRAEL S. The excretion of dextrose in the stomach and the small intestine, 274
KOLMER, JOHN A. Complement deviation in scarlet fever with comparative studies of the Wassermann and Noguchi systems, 235

LAMAR, RICHARD V. Chemo-immunological studies on localized infections. Third paper: Some further observations upon the action of certain soaps on the pneumococcus and its experimental infections, 256
LAMBERT, ROBERT A., and HANES, FREDERIC M. The cultivation of tissue in plasma from alien species, 129
——. The cultivation of tissues *in vitro* as a method for the study of cytotoxins, 453
Langerhans, islands of, hypertrophy and regeneration of, 500
Lepræ, Bacillus, the biochemistry of, 606
Leprosy, experimental immunity with reference to the bacillus of; a study of the factors determining infection in animals, 181
Lesions of the elastic tissue of the spleen and liver, 59
Lesions of the kidney, experimental, a study of the elimination of phenolsulphonephthalein in, 462
Lethal cardiac anaphylaxis in the rabbit, 476
LEVIN, ISAAC. Studies on immunity in cancers of the white rat. The significance of "the specific stroma reaction," 139
LEVIN, I., and STTENFIELD, M. J. On the mechanism of the formation of metastases in malignant tumors. An experimental study, 148
Light, interference bands of, formed by thin plates, the distribution and direction of motion of, as the thickness of the plate varies, 357
Liver and spleen, calcium and iron incrustation and other lesions of the elastic tissue of the, 59
Liver, proteins of the kidney and, 44

MARKS, HENRY K. Infection of rabbits with the virus of poliomyelitis, 116
MATTILL, H. A., and HAWK, P. B. A method for the quantitative determination of fecal bacteria, 433
Mechanism of chlorin retention in pneumonia, 289
Mechanism of the formation of metastases in malignant tumors, 148
MEDIGRECEANU, FLORENTIN. On the mechanism of chlorin retention in pneumonia, 289
——. On the composition of urinary albumin, 298
MELTZER, S. J. See AUER and MELTZER, 569
Meningitis, influenzal, serum treatment of, 73
Metastases in malignant tumors, the mechanism of the formation of, 148

Method for the pure cultivation of pathogenic *Treponema pallidum*, 99
Method for the quantitative determination of fecal bacteria, 433
Micrograph, a study of simultaneous tracings from the apex of the heart and the radial artery with the, 339
Micrograph, the presphygmic period with the, 520

NEPHRECTOMY, double, the ultimate result of, and the replantation of one kidney, 124
Nephritis, acute experimental: the elimination of nitrogen and chlorides, as compared with that of phenolsulphonephthalein, 366
Nephritis, experimental, due to bacterial poisons and cytotoxic sera, 306
Nerve fibrillæ in the pulmonary artery of the dog, 322
Nerve, vagus, action of, on the human heart, studies with the electrocardiograph on, 217
Nerve, vagus, effect of mechanical stimulation of, 217
NICHOLS, HENRY J. Further observations on certain features of experimental syphilis and yaws in the rabbit, 196
Nitrogen and chlorides, the elimination of, as compared with that of phenolsulphonephthalein in experimental acute nephritis, 366
NOGUCHI, HIDEYO. A method for the pure cultivation of pathogenic *Treponema pallidum* (*Spirochæta pallida*), 99
——. A cutaneous reaction in syphilis, 557
Noguchi and Wassermann systems, comparative studies of, in scarlet fever, 235
Normal sera, on the toxic action of certain, 25

OBSTRUCTIVE jaundice, experimental, 159
Optic thalamus, the relation of the, to respiration, circulation, temperature, and the spleen, 408

PALLIDUM, pathogenic *Treponema*, a method for the pure cultivation of, 99
Patching of the abdominal aorta with a piece of rubber, 126
Pathogenic *Treponema pallidum*, a method for the pure cultivation of, 99
Pathological pigment formation, the relation of hematin to, 612
Pathology of intraperitoneal bile injections in the rabbit, 445
PEARCE, L. See KING, BIGELOW, and PEARCE, 159
PEARCE, RICHARD M., and EISENBREY, A. B. A physiological study of experimental nephritis due to bacterial poisons and cytotoxic sera, 306
PEARCE, RICHARD M., KARSNER, HOWARD T., and EISENBREY, ARTHUR B. Studies in immunity and anaphylaxis. The proteins of the kidney and liver, 44
Pepsin, chemo-biological study of the relations of, to so-called anti-pepsin, 535
Phagocytic immunity in pneumococcus infections and in pneumonia with relation to the crisis, 109
Phenolsulphonephthalein, the elimination of, compared with that of nitrogen and chlorides in experimental acute nephritis, 366
Phenolsulphonephthalein, the elimination of, in various experimental lesions of the kidney, 462
Physiological study of experimental nephritis due to bacterial poisons and cytotoxic sera, 306
Pigment formation, pathological, the relation of hematin to, 612
Plasma from alien species, cultivation of tissue in, 129
Platelets, blood, the rate of regeneration of, 265
Pneumococcus and its experimental infections, the action of certain soaps on the, 256
Pneumococcus infections, phagocytic immunity in, 109
Pneumonia, phagocytic immunity in, 109
Pneumonia, the mechanism of chlorin retention in, 289
Poisons, bacterial, and cytotoxic sera, a physiological study of experimental nephritis due to, 306
Poliomyelitis, infection of rabbits with the virus of, 116
Precipitates, formation of, after the intravenous injection of salvarsan, 83
Precipitates, formation of, in the

blood *in vitro* by acid salvarsan solutions, 179
Presphygmic period, a study of, with the micrograph, 520
Pressure, differential, the status of respiration in the methods of, compared with that under the method of intratracheal insufflation, 569
Proteins of the kidney and liver, 44
Pulmonary artery of the dog, nerve fibrillæ in, 322
Pulmonary circulation, 326
Pulse, venous, time relations of heart sounds and, 594

RADIAL artery, a study of simultaneous tracings with the micrograph from the apex of the heart and the, 339
Reaction, cutaneous, in syphilis, 557
Reaction, specific stroma, the significance of, 139
Regeneration and hypertrophy of the islands of Langerhans, 500
Regeneration of blood platelets, the rate of, 265
Renal hypernephromas, malignant, the absence of adrenalin in, 550
Replantation of one kidney, the ultimate result of a double nephrectomy and, 124
Respiration, status of, in the methods of differential pressure compared with that under the method of intratracheal insufflation, 569
Respiration, the relation of the optic thalamus to, 408
RICHARDSON, MAURICE L. Biliary cirrhosis in the rabbit, 401
ROBINSON, G. CANBY, and DRAPER, GEORGE. Studies with the electrocardiograph on the action of the vagus nerve on the human heart. I. The effect of mechanical stimulation of the vagus nerve, 217
Rubber, patching of the abdominal aorta with a piece of, 126

SACHS, ERNEST. On the relation of the optic thalamus to respiration, circulation, temperature, and the spleen, 408
Salvarsan and acute anaphylaxis, 497
Salvarsan, effect of, upon the heart in dogs, 248
Salvarsan, formation of precipitates after the intravenous injection of, 83
Salvarsan solutions, acid, the formation of precipitates in the blood *in vitro* by, 179
Scarlet fever, complement deviation in, with comparative studies of the Wassermann and Noguchi systems, 235
Sera, cytotoxic, a physiological study of experimental nephritis due to bacterial poisons and, 306
Sera, the toxic action of certain normal, 25
Serum treatment of influenzal meningitis, 73
SITTENFIELD, M. J. See LEVIN and SITTENFIELD, 148
Soaps, the action of certain, on the pneumococcus and its experimental infections, 256
Specific stroma reaction, the significance of, 139
Spirochæta pallida, pathogenic, a method for the pure cultivation of, 99
Spleen and liver, calcium and iron incrustation and other lesions of the elastic tissue of the, 59
Spleen, on the relation of the optic thalamus to the, 408
SPRUNT, THOMAS P. Calcium and iron incrustation and other lesions of the elastic tissue of the spleen and liver, 59
STEWART, G. N. So-called biological tests for adrenalin in blood with some observations on arterial hypertonus, 377
Stimulation, mechanical, of the vagus nerve, studies with the electrocardiograph on, 217
Stomach, the excretion of dextrose in the, 274
Stroma reaction, specific, the significance of, 139
Strongyloides infections in man and animals in the Isthmian Canal Zone, 1
STROUSE, S. Phagocytic immunity in pneumococcus infections, and in pneumonia with relation to the crisis, 109
Syphilis, a cutaneous reaction in, 557
Syphilis and yaws, experimental, in the rabbit, 196

TECHNIQUE of the cultivation of tissues *in vitro*, 244
Temperature, the relation of the optic thalamus to, 408
TERRY, B. T. Intra-stomachal and

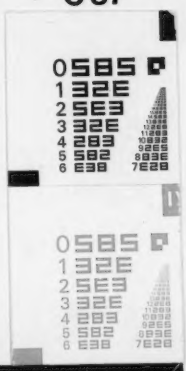
intra-intestinal inoculations of trypanosome virus with tests for immunity, 526
Tests, biological, so-called, for adrenalin in blood, with observations on arterial hypertonus, 377
Tests for immunity with intra-stomachal and intra-intestinal inoculations of trypanosome virus, 526
Thalamus, optic, the relation of, to respiration, circulation, temperature, and the spleen, 408
Time relations of the venous pulse and the heart sounds, 594
Tissue, cultivation of, in plasma from alien species, 129
Tissue, elastic, of the spleen and liver, 59
Tissues, cultivation of, *in vitro*, as a method for the study of cytotoxins, 453
Tissues, cultivation of, the technique of, 244
Toxic action of certain normal sera and its relation to anaphylaxis, 25
Tracings from the region near the apex of the heart, 351
Tracings, simultaneous, from the apex of the heart and the radial artery with the micrograph, 339
Treatment, serum, of influenzal meningitis, 73
Treponema pallidum, pathogenic, a method for the pure cultivation of, 99
Trypanosome virus, intra-stomachal and intra-intestinal inoculations of, with tests for immunity, 526
Tumors, malignant, the mechanism of the formation of metastases in, 148

URINARY albumin, composition of, 298

VAGUS nerve, action of, on the human heart, studies with the electrocardiograph on, 217
Vagus nerve, effect of mechanical stimulation of, 217
Venous pulse, time relations of heart sounds and, 594
Virus of poliomyelitis, infection of rabbits with, 116
Virus, trypanosome, intrastomachal and intra-intestinal inoculations of, with tests for immunity, 526

WASSERMANN and Noguchi systems, comparative studies of, in scarlet fever, 235
WOLLSTEIN, MARTHA. Serum treatment of influenzal meningitis, 73
WOOD, HORATIO C., JR. Studies of the pulmonary circulation, 326

YAWS and syphilis, experimental, in the rabbit, certain features of, 196

ZINSSER, HANS. On the toxic action of certain normal sera and its relation to anaphylaxis, 25

C37